

Surprising Enhancement of Fibrosis by Tubule-Specific Deletion of the TGF- β Receptor: A New Twist on an Old Paradigm

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Tubulointerstitial fibrosis has long been recognized as the most significant structural lesion associated with the development and progression of CKD. Since the pioneering work of Border and Ruoslahti,¹ the pleiotropic factor TGF- β has assumed a central role in the genesis of tubulointerstitial fibrosis. A multitude of studies have shown increased renal production of TGF- β in various CKD models and human biopsies of patients with CKD. Exogenous delivery or overexpression of TGF- β leads to the development of interstitial fibrosis, whereas a variety of strategies to inhibit TGF- β diminish the severity of experimentally induced renal disease.²

The development of interstitial fibrosis is a complicated process involving multiple different cell types contributing to scar formation.³ Because multiple cell types may either produce and/or be a target of TGF- β , the exact role of this factor in the fibrotic process is complex. TGF- β stimulates production of extracellular matrix (ECM) in multiple kidney cells types, including fibroblasts, pericytes, and mesangial cells as well as proximal tubule (PT) cells.^{3,4} In contrast, TGF- β has mixed effects on immune and inflammatory function, which may have variable effect on the development of renal fibrosis.⁴ Therefore, a more complete understanding of the TGF- β system requires analysis at the level of specific cell types.

Interest in the role of PT TGF- β in fibrosis derives, in part, from its prominent expression in these cells in response to injury. In addition to stimulation of ECM, TGF- β may induce PT growth arrest, apoptosis, expression of other fibrotic factors (e.g., connective tissue growth factor [CTGF]), and potentially, epithelial mesenchymal transition.⁴ Overexpression of TGF- β 1 using a PT-specific Tet-on promoter results in interstitial fibrosis,

in which the first lesions appear as an expansion of the peritubular interstitium.⁵ In addition, an elegant study by Hathaway *et al.*⁶ using an inducible transgenic approach to evaluate TGF- β 1 “dose response” in PT showed a strong correlation with TGF- β levels and interstitial fibrosis in diabetic Akita mice. These approaches highlight that PT is an important source of TGF- β contributing to fibrosis, but do not help clarify the role of tubular responses to TGF- β in this process.

In this issue of the *Journal of the American Society of Nephrology*, Nlandu-Khodo *et al.*⁷ investigate the role of PT-specific TGF- β signaling in the development of tubulointerstitial fibrosis. The authors used a PT-specific deletion of the TGF- β receptor type II (T β R II), the γ GT-Cre;Tgfr2 fl/fl mouse, in two well established models of progressive CKD: the aristolochic acid model and Ang II–induced hypertension. To their surprise, ablation of PT T β R II signaling exacerbated renal fibrosis in both models of CKD. The lack of PT T β R II was associated with sustained cellular injury as indicated by activation of apoptotic pathways and injury biomarkers, suggesting that T β R II activity is cytoprotective and that lack of PT stability may exacerbate fibrosis.

To explore why T β R II loss may activate fibrotic pathways, the investigators challenged T β R II -null PT cells to aristolochic acid stimulation *in vitro* and showed that these cells are predisposed to cell cycle arrest at G2/M. The identification of G2/M arrest represents an important mechanism potentially leading to fibrosis, as previous studies have reported that PT G2/M arrest was associated with fibrosis in several models of CKD.⁸ It is possible that T β R II null mice may have an enhanced proportion of PTs arrested at this phase of the cell cycle leading to increased fibrosis, but in the study by Nlandu-Khodo *et al.*,⁷ this was not directly evaluated in response to the aristolochic acid– or Ang II–induced injury *in vivo*.⁷

However, previous studies showed that human kidney PT cells arrest in G2/M after aristolochic acid–induced injury and manifest a profibrogenic phenotype as illustrated by enhanced expression of ECM genes, TGF- β , or CTGF (Figure 1).⁸ In mouse PT cells lacking T β R II , G2/M arrest was associated with enhanced CTGF expression (but not TGF- β 1) versus control cells,⁷ suggesting that T β R II signaling preserves cell cycle activity, preventing the induction of profibrotic molecules.

Studies were also conducted to understand the molecular signaling pathway responsible for impaired PT activity in the absence of T β R II . Because T β R II signals through multiple different intracellular signaling pathways,⁴ RNAseq was used to evaluate gene expression in injured PT cells lacking T β R II . The most significant alterations were associated with the Wnt signaling pathway. The investigators further showed that β -catenin, an important factor in Wnt signaling, showed impaired nuclear localization in the absence of T β R II . Interestingly, the GSK3 inhibitor BIO (6-bromoindirubin-3'-oxime) was used to enhance β -catenin activity in T β R II knockout PT, which reduced G2/M arrest, apoptosis, and the expression of CTGF.

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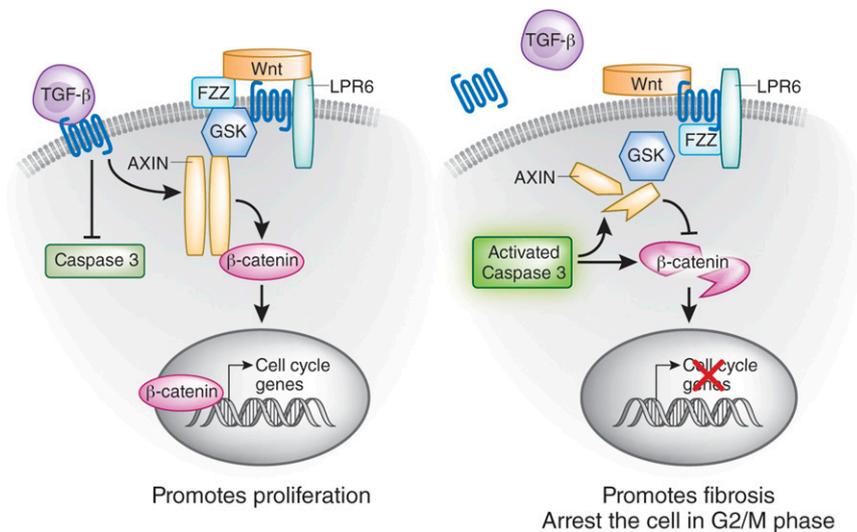


Figure 1. TGF- β receptor signaling is proposed to facilitate successful recovery from cellular damage by interacting with the Wnt- β -catenin pathway. Genetic ablation of the TGF- β receptor in proximal tubule inhibits normal nuclear localization of β -catenin resulting in increased death, cell cycle arrest and expression of fibrotic factors.

Finally, the investigators crossed γ GT-Cre;Tgfr2 fl/fl with *Ctnnb1*(ex3)fl/fl, mice, a strategy designed to introduce stabilized expression of β -catenin in PT lacking the T β RII. This strategy restored Wnt-dependent signaling in T β RII knockout cells and significantly attenuated the fibrotic response to aristolochic acid treatment in transgenic mice lacking PT T β RII. Therefore, T β RII signaling in response to injury results in cytoprotection in a Wnt/ β -catenin-dependent fashion.

In addition to impressive data and experimental design, an important conclusion from this study is that both overexpression of TGF- β and inhibition of T β RII signaling can evoke interstitial fibrosis. The authors note that such contrasting observations using genetic approaches provide extreme scenarios of either increases or decreases in expression and illustrate the principal that either too much or too little TGF- β is deleterious.

The study also illustrates an important perspective with regard to the underlying nature of TGF- β as a factor promoting tissue repair by influencing activities, such as cell proliferation, differentiation, ECM production, and immune cell infiltration. In a field dominated by fibrotic disease, the nephrology community has focused primarily on the “Dark Side of Tissue Repair,” in which continued injurious stimulation (for example, secondary to diabetes or hypertension) may lead to excessive scar formation.^{1,3} This study, therefore, highlights what is often recognized but generally overlooked (*i.e.*, TGF- β is an essential factor in normal homeostatic cellular repair, and its induction represents a normal adaptive response).

Clearly, TGF- β remains an important target to reduce the progression of CKD, but whether direct TGF- β antagonism represents a long-term treatment strategy is not known. The lack of significant effect on CKD progression in a recently published clinical trial using TGF- β antibodies in patients with diabetes represents a significant setback for this approach.⁹ The reasons for the failure of this trial are not clear, but it is possible

that sustained inhibition of TGF- β for several months may indicate that the treatment has become refractory. Additionally, it could be speculated that chronic TGF- β antagonism may negatively affect PT repair. For these reasons, it may be interesting to determine if TGF- β neutralization would mitigate the development of fibrosis in PT T β RII knockout mice with impaired tubular function. In such a case, one might determine if an alternative fibrotic pathway, such as CTGF, from G2/M arrested cells is sufficient to drive the fibrotic process.

Wnt signaling is thought to be important in fibrosis in not only kidney but also, lung and liver. However, the molecular pathways leading to fibrosis are not clearly defined. Inhibition of Wnt is known to effectively reduce TGF- β activity and the induction of downstream profibrotic genes, such as Col1, α -SMA, and fibronectin.^{1,3} β -Catenin has been shown to be associated with nephrogenesis and tubular repair after acute injury and is also associated with renal fibrosis in CKD models; therefore, like TGF- β , it is deleterious if expression is sustained.¹⁰

As a result, it is tempting to speculate that targeting Wnt/ β -catenin signaling may be a potential target in treatment of fibrosis. Recent studies indicate that GSK3 inhibitors minimized the development of renal fibrosis in models of AKI or diabetic kidney injury,^{11,12} although the direct effect of these treatments on β -catenin was not addressed in these studies. There are 19 different Wnt ligands signaling *via* noncanonical and/or canonical pathways, thereby limiting the efficacy of β -catenin/GSK inhibitors. Madan *et al.*¹³ have shown that targeting Porcupine, an *O*-acyl transferase, which influences binding of Wnt to all its ligands, effectively reduced nuclear localization of β -catenin as well as fibrosis in mouse model of UO. In addition, porcupine inhibition also blunts the proliferation of fibroblasts and the expression of key proinflammatory cytokines, which are, in turn, capable of eliciting Wnt generation.

However, in the article by Nlandu-Khodo *et al.*,⁷ the authors highlight the importance of the proper balance in Wnt-activated β -catenin signaling as a target of TGF- β signaling in injured PT cells influencing progress through the cell cycle. Thus, like TGF- β , the influence of Wnt/ β -catenin activation is context dependent. In this regard, it is worth noting that, in a study using a similar strategy for PT-specific ablation, Zhou *et al.*¹⁴ showed that PT ablation of β -catenin also exacerbated renal injury, similar to the results obtained with T β R11.

In summary, the study by Nlandu-Khodo *et al.*⁷ has shown the importance T β R11-induced Wnt signaling in the progression of renal fibrosis. Multiple small molecule Wnt inhibitors have been developed and are in phase 1 clinical trials to treat Wnt-driven cancers.¹⁵ Although such agents could be used in targeting fibrosis, this study necessitates that normal homeostatic cellular functions be considered if such studies are pursued.

DISCLOSURES

None.

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See related article, "Blocking TGF- β and β -Catenin Epithelial Crosstalk Exacerbates CKD," on pages 3490–3503.

Obesity-Related CKD: When Kidneys Get the Munchies

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A brief walk through a typical renal outpatient clinic or dialysis unit may give rise to a suspicion of a link between society's dual public health problems of obesity and CKD. Indeed, epidemiologic studies have consistently found obesity to be an important risk factor for CKD, and it remains significant even after correcting for other obvious coassociations, such as hypertension and diabetes.^{1,2} This effect of obesity in increasing the risk of CKD is seen in all age groups and implicated across the full spectrum of CKD spanning from early CKD to ESRD. Furthermore, obesity accelerates renal injury caused by other diseases, such as GN (Figure 1).

Despite the epidemiologic links between obesity and CKD, obesity is rarely considered as a distinct cause of CKD in humans. This is partly due to the lack of a generally identifiable pattern of injury linking obesity with CKD. Histologically, the pattern that is generally ascribed to obesity is that of obesity-related glomerulopathy (ORG), which consists predominantly of focal glomerulosclerosis and podocytopeny.³ However, ORG is mainly associated with morbid obesity, and although an important entity, the number of patients with diagnosed cases is low and clearly insufficient to explain the broader

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