Action Mechanisms and Therapeutic Targets of Renal Fibrosis

Shi-Xing Ma¹, You-Quan Shang¹, Huan-Qiao Zhang¹, Wei Su¹,*

¹Department of Nephrology, Baoji Central Hospital, No. 8 Jiangtan Road, Baoji, Shaanxi 721008, China

Abstract
Renal fibrosis was a chronic and progressive process affecting kidneys in chronic kidney disease (CKD), regardless of cause. Although no effective targeted therapy yet existed to retard renal fibrosis, a number of important recent advances have highlighted the cellular and molecular mechanisms underlying the renal fibrosis. The advances including TGF-β/Smad pathway, oxidative stress and inflammation, hypoxia and gut microbiota-derived from uremic solutes were highlighted that could provide therapeutic targets. New therapeutic targets and strategies that are particularly promising for development of new treatments for patients with CKD were also highlighted.

Corresponding author: Wei Su, MD, Department of Nephrology, Baoji Central Hospital, No. 8 Jiangtan Road, Baoji, Shaanxi 721008, China, E-mail: suwei831@foxmail.com

Keywords: chronic kidney disease; renal fibrosis; TGF-β/Smad pathway; oxidative stress and inflammation; hypoxia; gut microbiota; uremic solutes.

Received: Oct 26, 2018 Accepted: Nov 04, 2018 Published: Nov 12, 2018

Editor: Ying-Yong Zhao, Northwest University, China.
Introduction

Chronic kidney disease (CKD) had a high prevalence all over the world and was closely associated with high mortality [1-3]. The prevalence of CKD was estimated to be 8-16% worldwide. In patients over 64 year old, the prevalence elevated to 23.4-35.8%, indicating increasing age contributed to elevate CKD. The yearly economic costs of medicine care for patients with CKD and or end-stage renal disease over age 65 were $60 billion, representing 24% of total Medicare expenditures in 2011 in America. According to the Kidney Disease Outcomes Quality Initiative, the international guidelines define and classify CKD as decreased renal function shown by glomerular filtration rate (GFR) of less than 60 mL/min per 1.73 m², or markers of kidney damage, or both, of at least three months duration, regardless of underlying cause [1]. CKD were divided into five stages as follow: Stage 1: Kidney damage (pathological abnormalities or markers of damage including abnormalities in blood or urine tests or in imaging studies) with normal or raised glomerular filtration rate (≥90 mL per min per 1.73 m²); Stage 2: Glomerular filtration rate 60–89 mL per min per 1.73 m² with evidence of kidney damage; Stage 3: Glomerular filtration rate 30–59 mL per min per 1.73 m²; Stage 4: Glomerular filtration rate 15–29 mL per min per 1.73 m²; Stage 5: End-stage renal failure; glomerular filtration rate <15 mL per min per 1.73 m² [1,4,5].

Renal fibrosis was characterized as a common endpoint of diverse CKD which resulted in functional damage ultimately leading to terminal renal failure [6-9]. Renal fibrosis is generally regarded as the dark side of tissue repair mechanisms. Fibrogenesis might be involved in the tubulointerstitial resulting in tubulointerstitial fibrosis, glomeruli resulting in glomerulosclerosis or the arterial vasculature resulting in atherosclerotic lesions [5,10]. Various action mechanisms were implicated in renal diseases and renal fibrosis [11-18]. Knowledge of the complex pathophysiological mechanisms contributed to CKD remains limited. In this review, we verify the critical roles of transforming growth factor-β (TGF-β)/Smad pathway, oxidative stress and inflammation, hypoxia and gut microbiota-derived from uremic solutes in the pathophysiology of CKD and renal fibrosis, summarize the action mechanisms of renal fibrosis, and discuss the effects of these mediators in the context of renal fibrosis.

TGF-β/Smad in Renal Fibrosis

TGF-β was essential for normal tissue development, repair and maintenance for organ functions. TGF-β1 was known as an antiinflammation cytokine [18]. It produced anti-inflammatory effects through inhibition of mitogenesis and cytokine responses in glomerular cells and inhibiting infiltrating cells [18]. Transforming growth factor-β (TGF-β1) knockout mice showed multi-organ inflammation and TGF-β1 deficient mice exhibited lethal inflammation and die within three weeks [19]. Similarly, deletion of TGF-β1 or transforming growth factor-β receptor type II (TGFβRII) genes has been shown to cause autoimmune diseases [20,21]. Mice over-expressing latent TGF-β1 were protected against inflammation and renal fibrosis in obstructive nephropathy and glomerulonephritis [21-23]. Although TGF-β-induced inhibition of NF-κB-mediated renal inflammation through induction of Smad7-dependent renal inflammation has been recently demonstrated [21,23], the signaling action mechanisms of its anti-inflammatory action remain unclear. Yet, upregulated TGF-β1 was related to pathological disorders in many kidney diseases [24,25].

There is extensive evidence pointing to TGF-β1 upregulation and its role in the pathogenesis of renal fibrosis in both animal models and patients with CKD [18,26]. TGF-β1 mediated progressive renal fibrosis by stimulating production and suppressing degradation of extracellular matrix (ECM). Moreover, TGF-β1 caused renal fibrosis by the transformation of tubular epithelial cells to myofibroblasts through epithelial-to-mesenchymal transition (EMT) [23]. The central role of TGF-β1 on EMT and renal fibrosis has been confirmed by many experiments which indicated the ability of TGF-β1 blockade with decorin, neutralizing TGF-β antibody or anti-sense oligonucleotides to attenuate renal fibrosis [18]. Direct evidence for the causal role of TGF-β1 in renal fibrosis is confirmed in mice over-expressing an active TGF-β1 form [27]. TGF-β has been shown to serve a critical mediator in the pathogenesis of glomerulosclerosis in patients with glomerular diseases, such as lupus nephritis, immunoglobulin A nephropathy, membranous nephropathy, focal and segmental glomerulosclerosis.
and diabetic nephropathy. The upregulation of the three TGF-β isoforms and TGFβRI and TGFβRII has been uncovered in the glomeruli and tubulointerstitium in kidney diseases [28]. Upregulation of TGF-β1 caused excessive ECM productions, reduced ECM-degrading proteinase activity and upregulated proteinase inhibitor, that resulted in excessive ECM deposition. In progressive podocyte-associated glomerular diseases, excessive TGF-β1 expression in the podocytes has been indicated the role of TGF-β1 in podocyte injury in patients with IgA nephropathy, focal and segmental glomerulosclerosis (FSGS) and diabetic nephropathy [29]. Tubular and glomerular TGF-β expression was increased in early and late stages of diabetic nephropathy and inversely correlates with glycemic control in diabetic patients [30]. TGF-β1 expression was stimulated by glomerular stretch and hyperglycemia in early stage, and by angiotensin II, advanced glycation end-product and platelet-derived growth factor [30]. Angiotensin II has been demonstrated to raise expression of TGF-β1 and its receptors [31,32].

Mounting studies have identified Smad2/3 as two major downstream mediators of the actions of TGF-β1 (Figure 1). In the context of renal fibrosis, Smad2/3 are activated in both patients and animal models with CKD of diverse etiologies such as hypertensive nephropathy [31,33,34], obstructive kidney disease [35], remnant kidney disease [36,37], chronic renal allograft injury [38], diabetic nephropathy [39-41] and drug-induced nephropathy [42]. Many fibrogenic genes including plasminogen activator inhibitor-1, tissue inhibitor of metalloproteinase-1, connective tissue growth factor, proteoglycans, integrins and collagens have been shown to be the downstream targets of TGF-β1/Smad3 signaling [43]. These observations demonstrate the central role of Smad3 in TGF-β1/Smad signaling-mediated renal fibrosis.

Many natural products have been widely used as anti-fibrotic agents [44-56]. Poricoic acid ZC, Poricoic acid ZD and poricoic acid ZE, isolated from the surface layer of Poria cocos, exhibited a strong inhibitory effect on renal fibrosis and podocyte injury. The findings showed that new RAS inhibitors poricoic acid ZC, poricoic acid ZD and poricoic acid ZE treatment significantly attenuated EMT production by inhibiting Wnt/β-catenin pathway activation and specific Smad3 phosphorylation by blocking the interaction of TGFβRI with Smad3 signaling in both TGF-β1- and angiotensin II-treated HK-2 cells as well as unilateral ureteral obstruction (UUO) mice [57]. Similarly, renal fibrosis in a variety of animal models were mitigated via TGF-β1/Smad pathways by administration of natural products, such as GQ5 [58], curcumin [59,60], arctigenin [61], resveratrol [62], sinomenine [63], berberine [64,65], leonurine [66], rutin [67], bergenin [68], oxymatrine [69,70], oleancolic acid [71], tanshinone IIA [72], astragaloside IV [73,74], (+/-)-sinensilactam A [75] and epigallocatechin-3-gallate [76].

**Oxidative Stress and Inflammation and Renal Fibrosis**

Oxidative stress and inflammation played a central part in the pathogenesis and progression of CKD [77-82]. Renal fibrosis was a relatively common cause of CKD in humans. Rats or mice fed an adenine-containing diet exhibited severe renal fibrosis resembling that seen in humans [83]. The renal fibrosis in this model was mediated by the renal tubular precipitation of dihydroxyadenine resulting in interstitial inflammatory cell infiltration, tubular epithelial cell injury, fibrosis and progressive deterioration of kidney function [84]. Progressive renal disease was largely driven by inflammation and oxidative stress. Oxidative stress and inflammation were inseparably linked as they produced a vicious cycle in which oxidative stress triggered inflammation by many mechanisms including activation of the transcription factor kappa B which resulted in the activation and recruitment of immune cells [7] (Figure 1). Inflammation, in turn, triggered oxidative stress through production of reactive oxygen species and reactive nitrogen species by the activated leukocytes and resident cells. Together these events promote tissue damage by inducing apoptosis, necrosis and fibrosis [85].

Under physiological conditions, oxidative stress gives rise to upregulation of the endogenous antioxidant and cytoprotective proteins and enzymes to prevent tissue injury. This process was mediated by the activation of the Nrf2 which regulated the basal activity and coordinated induction of numerous genes that encode various antioxidant and phase 2 detoxifying
enzymes and related proteins [81]. Nrf2 is an inactive complex in the cytoplasm by the repressor molecule, Keap1 which facilitated its ubiquitination (Figure 1). Keap1 contained reactive cysteine residues which function as intracellular redox sensors. Nuclear translocation of Nrf2 occurred by phosphorylation of its threonine or serine residues via upstream kinases, such as mitogen-activated protein kinases, protein kinase C, phosphatidylinositol-3-kinase/Akt, casein kinase-2 and PKR-like ER kinase [78]. Regulation of cellular antioxidant and anti-inflammatory machinery by Nrf2 plays a central part in defense against oxidative stress. A number of studies have reported that the imbalance between NF-κB and Nrf2 pathways contributed to CKD and renal fibrosis [63,77,80,81,86].

Besides, inflammation could in the activation of immune cells, including macrophages, dendritic cells and T cells. These immune cells release profibrotic cytokines and growth factors that contribute to renal fibrosis [87,88].

Hypoxia and Renal Fibrosis

The kidney was physiologically hypoxic despite its plentiful blood supply, because an oxygen shunt is
present between arteries and veins. Therefore, it is reasonable to consider that erythropoietin-producing cells reside in the kidney, where they can sensitively detect hypoxia owing to anemia [89-91]. Physiological hypoxia has been uncovered in mammals and in hypoxia-monitoring transgenic mice and rats produced by using hypoxia-inducible factor system. Expansive kidney hypoxia in CKD has also been verified in both patients and animal models. In CKD, hypoxia appeared in tubulointerstitium via multiple mechanisms. First, glomerulosclerosis resulted in a reduction of flow in downstream peritubular capillary, which further compromised by constriction of efferent arterioles of glomeruli and peritubular capillary owing to RAS activation. Second, the loss of peritubular capillaries occurred owing to fibrosis reduced blood perfusion. Third, excessive ECM deposition by fibrogenesis increased the distance between capillary and tubular, diminishing the efficiency of oxygen diffusion.

Upregulation of hypoxia-inducible factor 1α (HIF-1α) in transgenic mice upregulated vascular endothelial growth factor and platelet-derived growth factor-B expression and augmented endothelial cell proliferation. Although increased production and decreased deposition of ECM were observed in transgenic mice compared to control mice, they did not display renal injury or dysfunction [92]. These results were consistent with study indicating that conditional knockout of HIF-1α in the proximal tubules lessened fibrosis in mouse UUO [89]. Given that deposition of ECM was a part of repair processes unless it is uncontrolled, hypoxia-inducible factor activation by hypoxia in tubular cells mitigated renal injury by the upregulation of angiogenic and fibrogenic factors.

**Uremic Solutes and Renal Fibrosis**

Fibrosis was the final result of a complex signaling cascade of intracellular and intercellular and molecular responses initiated by organ injury [10]. The fibrotic process and fibrotic-associated pathways are conserved between different organs. EMT has emerged as a mainly origin of collagenous matrix-producing myofibroblasts that contributed to the fibrotic response [46,93-95]. Renal fibrosis ends in uremic stage, yet uremia per se also further promoted the fibrogenesis owing to the direct biological effects of uremic toxins, such as, indoxyl sulfate (IS) and p-cresyl sulfate (p-CS). At least five uremic toxins showed a direct link to EMT and renal fibrosis [96-99].

Uremic toxin IS was a small organic aromatic polycyclic anion derived from dietary tryptophan by gut microbiota that has widely been investigated in linking with CKD-associated cardiovascular disease [96,100-102], and IS can induce vascular calcification and correlates with coronary artery disease and mortality [103]. IS also contributed to a plethora of pathologies observed in dialysis patients, including tubulointerstitial inflammation and kidney damage [96]. IS overload augmented the gene expression of tissue inhibitor of metalloproteinases-1, intercellular adhesion molecule-1, alpha-1 type I collagen, and TGF-β in the renal cortex of 5/6 nephrectomized rats [104]. Moreover, IS stimulated the production of TGF-β in renal proximal tubular cells. Other study indicated that stimulation of HK-2 cells to IS resulted in a reactive oxygen species-mediated upregulation of plasminogen activator inhibitor-1, a downstream signaling mediator of the TGF-β signaling related to most aggressive kidney diseases [105]. Furthermore, another study demonstrated that IS can increase α-SMA and TGF-β expression in HK-2 cells by activation of the (pro)renin receptor through reactive oxygen species-Stat3-NF-κB pathways [106]. IS also activated the TGF-β signaling, as showed by an increased Smad2/3 phosphorylation [97,107].

Although EMT contribution to fibrosis was controversial, phenotypic alterations reminiscent of EMT, also presented as epithelial phenotypic changes, might play an important role in the fibrogenesis and disease progression [108]. A number of studies have demonstrated that IS induced EMT, as indicated by a downregulated expression of E-cadherin and zona occludens-1, and upregulated α-smooth muscle actin (α-SMA) expression in rat proximal tubular cells (NRK-52E) and rat kidneys [109]. Furthermore, IS promoted EMT-associated transcription factor Snail expression, concurrent with an elevated expression of α-SMA and fibronectin and diminished E-cadherin expression in vitro [97]. Similar effects of IS have also been observed in human renal cell models [109].

In addition, genetic or microRNA-based mechanisms are also reported to inhibit renal fibrosis.
through modulating signaling pathways to prevent the progression of renal fibrosis during CKD. Knockdown of profibrotic factor Smad4 alleviated renal fibrosis in mice [110]. microRNA-23b, microRNA-30e and microRNA -135a was significantly altered in CKD mice [111,112], indicating microRNAs as biomarkers and therapeutic targets for CKD.

**Conclusion**

From the above it is clear that our knowledge of action mechanisms contributing to renal fibrosis had rapidly investigated and expanded over the several decades but we were still confused. Nevertheless, the novel knowledge obtained recently points to many new methods to combat renal fibrosis, at least partial reversal of fibrotic tubulointerstitial injury. Future investigation need to clarify whether individual mechanism contributes to all or at least many renal fibrosis models and would therefore be main candidates for therapeutic strategy and intervention at very early stage of fibrogenesis.

**Conflict of Interest**

The authors declare that there is no conflict of interest.

**References**

1. Webster AC, Nagler EV, Morton RL, Masson P. Chronic kidney disease. Lancet 2017; 389: 1238-1252.
2. Hocher B, Adamski J. Metabolomics for clinical use and research in chronic kidney disease. Nat Rev Nephrol 2017; 13: 269-284.
3. Zhang L, Long J, Jiang W, et al. Trends in chronic kidney disease in China. The New England journal of medicine 2016; 375: 905-906.
4. Stevens LA, Coresh J, Greene T, Levey AS. Assessing kidney function--measured and estimated glomerular filtration rate. The New England journal of medicine 2006; 354: 2473-2483.
5. Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. Nature medicine 2012; 18: 1028-1040.
6. Meng XM, Tang PMK, Li J, Lan HY. TGF-β/Smad signaling in renal fibrosis. Front Physiol 2015; 6: 82.
7. Lan HY. Diverse roles of TGF-beta/Smads in renal fibrosis and inflammation. International journal of biological sciences 2011; 7: 1056-1067.
8. Loboda A, Sobczak M, Jozkowicz A, Dulak J. TGF-beta1/Smads and miR-21 in renal fibrosis and inflammation. Mediators of inflammation 2016; 2016: 8319283.
9. Higgins SP, Tang Y, Higgins CE, et al. TGF-beta1/ p53 signaling in renal fibrogenesis. Cellular signalling 2018; 43: 1-10.
10. Hu HH, Chen DQ, Wang YN, et al. New insights into TGF-β/Smad signaling in tissue fibrosis. Chem Biol Interact 2018; 292: 76-83.
11. Afandi B, Bernieh B. Acute bilateral hydro nephrosis after the use of dapagliflozin. Journal of Nephrology Advances 2015; 1: 42-47.
12. Andreucci M, Faga T, De Sarro G, Michael A. The toxicity of iodinated radiographic contrast agents in the clinical practice. Journal of Nephrology Advances 2015; 1: 6-41.
13. Ehsan A, Lone A, Sabir O, Tareef N, Riaz S, Tanvir I. Refractory anaemia with hyperoxalurea. Journal of Nephrology Advances 2015; 1: 1-5.
14. Soni SS, Barnela SR, Saboo SS, Deshpande AV, Deshmukh SS, Takalkar UV. Arteriovenous Fistula in A Patient with Aberrant Radial Artery. Journal of Nephrology Advances 2015; 1: 6-41.
15. G. EB, Dami F, Hanin H, Kabbali N, Arrayhani M, Sqalli HT. Bedside lung ultrasound in the assessment of volume status in chronic hemodialysis patients. Journal of Nephrology Advances 2015; 1: 48-57.
16. Edeling M, Ragig S, Huang S, Pavenstadt H, Susztak K. Developmental signalling pathways in renal fibrosis: the roles of Notch, Wnt and Hedgehog. Nat Rev Nephrol 2016; 12: 426-439.
17. Kok HM, Falke LL, Goldschmeding R, Nguyen TQ. Targeting CTGF, EGF and PDGF pathways to prevent progression of kidney disease. Nat Rev Nephrol 2014; 10: 700-711.
18. Meng XM, Nikolic-Paterson DJ, Lan HY. TGF-β: the master regulator of fibrosis. Nat Rev Nephrol 2016; 12: 325-338.
19. Yaswen L, Kulkarni AB, Fredrickson T, et al. Autoimmune manifestations in the transforming
growth factor-β1 knockout mouse. Blood 1996; 87: 1439-1445.

20. Li MO, Sanjabi S, Flavell RA. Transforming growth factor-β controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and -independent mechanisms. Immunity 2006; 25: 455-471.

21. Li MO, Wan YY, Flavell RA. T cell-produced transforming growth factor-β1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation. Immunity 2007; 26: 579-591.

22. Huang XR, Chung AC, Wang XJ, Lai KN, Lan HY. Mice overexpressing latent TGF-β1 are protected against renal fibrosis in obstructive kidney disease. American journal of physiology Renal physiology 2008; 295: F118-127.

23. Huang XR, Chung AC, Zhou L, Wang XJ, Lan HY. Latent TGF-β1 protects against crescentic glomerulonephritis. Journal of the American Society of Nephrology : JASN 2008; 19: 233-242.

24. Budi EH, Duan D, Derynck R. Transforming Growth Factor-β Receptors and Smads: Regulatory Complexity and Functional Versatility. Trends in cell biology 2017; 27: 658-672.

25. Sureshbabu A, Muhsin SA, Choi ME. TGF-β signaling in the kidney: profibrotic and protective effects. American journal of physiology Renal physiology 2016; 310: F596-f606.

26. Lan HY. Diverse roles of TGF-β/Smads in renal fibrosis and inflammation. International journal of biological sciences 2011; 7: 1056-1067.

27. Vanhove T, Goldschmeding R, Kuypers D. Kidney fibrosis: origins and interventions. Transplantation 2017; 101: 713-726.

28. Shi Y, Massague J. Mechanisms of TGF-β signaling from cell membrane to the nucleus. Cell 2003; 113: 685-700.

29. Sharma K. Obesity, oxidative stress, and fibrosis in chronic kidney disease. Kidney Int Suppl (2011) 2014; 4: 113-117.

30. Sharma K, McGowan TA. TGF-β in diabetic kidney disease: role of novel signaling pathways. Cytokine & growth factor reviews 2000; 11: 115-123.

31. Liu Z, Huang XR, Chen HY, Fung E, Liu J, Lan HY. Deletion of angiotensin-converting enzyme-2 promotes hypertensive nephropathy by targeting Smad7 for ubiquitin degradation. Hypertension (Dallas, Tex : 1979) 2017; 70: 822-830.

32. Nogueira A, Pires MJ, Oliveira PA. Pathophysiological mechanisms of renal fibrosis: A review of animal models and therapeutic strategies. In vivo (Athens, Greece) 2017; 31: 1-22.

33. Wang W, Huang XR, Canlas E, et al. Essential role of Smad3 in angiotensin II-induced vascular fibrosis. Circulation research 2006; 98: 1032-1039.

34. Liu GX, Li YQ, Huang XR, et al. Smad7 inhibits AngII-mediated hypertensive nephropathy in a mouse model of hypertension. Clinical science (London, England : 1979) 2014; 127: 195-208.

35. Zhou B, Mu J, Gong Y, et al. Brd4 inhibition attenuates unilateral ureteral obstruction-induced fibrosis by blocking TGF-β-mediated Nox4 expression. Redox biology 2017; 11: 390-402.

36. Yang F, Huang XR, Chung AC, Hou CC, Lai KN, Lan HY. Essential role for Smad3 in angiotensin II-induced tubular epithelial-mesenchymal transition. The Journal of pathology 2010; 221: 390-401.

37. Huang XZ, Wen D, Zhang M, et al. Sirt1 activation ameliorates renal fibrosis by inhibiting the TGF-β/Smad3 pathway. Journal of cellular biochemistry 2014; 115: 996-1005.

38. Wang YY, Jiang H, Pan J, et al. Macrophage-to-myofibroblast transition contributes to interstitial fibrosis in chronic renal allograft Injury. Journal of the American Society of Nephrology : JASN 2017; 28: 2053-2067.

39. Chung AC, Zhang H, Kong YZ, et al. Advanced glycation end-products induce tubular CTGF via TGF-β-independent Smad3 signaling. Journal of the American Society of Nephrology : JASN 2010; 21: 249-260.

40. Chen HY, Huang XR, Wang W, et al. The protective role of Smad7 in diabetic kidney disease: mechanism and therapeutic potential. Diabetes 2011; 60: 590-601.

41. Al-Rasheed NM, Al-Rasheed NM, Al-Amin MA, et al.
Fenofibrate attenuates diabetic nephropathy in experimental diabetic rat's model via suppression of augmented TGF-β1/Smad3 signaling pathway. Archives of physiology and biochemistry 2016; 122: 186-194.

42. Zhou L, Fu P, Huang XR, et al. Mechanism of chronic aristolochic acid nephropathy: role of Smad3. American journal of physiology Renal physiology 2010; 298: F1006-1017.

43. Verrecchia F, Chu ML, Mauviel A. Identification of novel TGF-β/Smad gene targets in dermal fibroblasts using a combined cDNA microarray/promoter transactivation approach. The Journal of biological chemistry 2001; 276: 17058-17062.

44. Chen DQ, FengYL, Cao G, Zhao YY. Natural products as a source for antifibrosis therapy. Trends in pharmacological sciences 2018; 39: 937-952.

45. Chen DQ, Hu HH, Wang YN, FengYL, Cao G, Zhao YY. Natural products for the prevention and treatment of kidney disease. Phytomedicine 2018; 50: 50-60.

46. Davis FM, Stewart TA, Thompson EW, Monteith GR. Targeting EMT in cancer: opportunities for pharmacological intervention. Trends in pharmacological sciences 2014; 35: 479-488.

47. Jiang WY. Therapeutic wisdom in traditional Chinese medicine: a perspective from modern science. Trends in pharmacological sciences 2005; 26: 558-563.

48. Zhang ZH, Li MH, Liu D, et al. Rhubarb protect against tubulointerstitial fibrosis by inhibiting TGF-β/Smad pathway and improving abnormal metabolome in chronic kidney disease. Front Pharmacol 2018; 9:

49. Dou F, Miao H, Wang JW, et al. An integrated lipidomics and phenotype study reveals protective effect and biochemical mechanism of traditionally used Alisma orientale Juzepzuk in chronic renal disease. Front Pharmacol 2018; 9: 53.

50. Zhang ZH, Vaziri ND, Wei F, Cheng XL, Bai X, Zhao YY. An integrated lipidomics and metabolomics reveal nephroprotective effect and biochemical mechanism of Rheum officinale in chronic renal failure. Sci Rep 2016; 6: 22151.

51. Zhang ZH, Wei F, Vaziri ND, et al. Metabolomics insights into chronic kidney disease and modulatory effect of rhubarb against tubulointerstitial fibrosis. Sci Rep 2015; 5: 14472.

52. Chen H, Yang T, Wang MC, Chen DQ, Yang Y, Zhao YY. Novel RAS inhibitor 25-O-methylalisol F attenuates epithelial-to-mesenchymal transition and tubulo-interstitial fibrosis by selectively inhibiting TGF-β-mediated Smad3 phosphorylation. Phytomedicine 2018; 42: 207-218.

53. Wang M, Chen DQ, Wang MC, et al. Poricoic acid ZA, a novel RAS inhibitor, attenuates tubulo-interstitial fibrosis and podocyte injury by inhibiting TGF-β/Smad signaling pathway. Phytomedicine 2017; 36: 243-253.

54. Wang M, Chen DQ, Chen L, et al. Novel RAS inhibitors poricoic acid ZG and poricoic acid ZH attenuate renal fibrosis via Wnt/β-catenin pathway and targeted phosphorylation of smad3 signaling. Journal of agricultural and food chemistry 2018; 66: 1828-1842.

55. Zhao YY, Zhang L, Long FY, et al. UPLC-Q-TOF/HSMS/MS(E)-based metabolomics for adenine-induced changes in metabolic profiles of rat faeces and intervention effects of ergosta-4,6,8(14),22-tetraen-3-one. Chem Biol Interact 2013; 201: 31-38.

56. Chen L, Chen DQ, Wang M, et al. Role of RAS/Wnt/β-catenin axis activation in the pathogenesis of podocyte injury and tubulo-interstitial nephropathy. Chem Biol Interact 2017; 273: 56-72.

57. Wang M, Chen DQ, Chen L, et al. Novel inhibitors of the cellular renin-angiotensin system components, poricoic acids, target Smad3 phosphorylation and Wnt/beta-catenin pathway against renal fibrosis. Br J Pharmacol 2018; 175: 2689-2708.

58. Ai J, Nie J, He J, et al. GQ5 hinders renal fibrosis in obstructive nephropathy by selectively inhibiting TGF-β-induced Smad3 phosphorylation. J Am Soc Nephrol 2015; 26: 1827-1838.

59. Sun X, Liu Y, Li C, et al. Recent advances of Curcumin in the prevention and treatment of renal fibrosis. BioMed research international 2017; 2017: 2418671.
60. Zhou X, Zhang J, Xu C, Wang W. Curcumin ameliorates renal fibrosis by inhibiting local fibroblast proliferation and extracellular matrix deposition. Journal of pharmacological sciences 2014; 126: 344-350.

61. Li A, Zhang X, Shu M, et al. Arctigenin suppresses renal interstitial fibrosis in a rat model of obstructive nephropathy. Phytomedicine : international journal of phytotherapy and phytopharmacology 2017; 30: 28-41.

62. Chen CL, Chen YH, Tai MC, Liang CM, Lu DW, Chen JT. Resveratrol inhibits transforming growth factor-β2-induced epithelial-to-mesenchymal transition in human retinal pigment epithelial cells by suppressing the Smad pathway. Drug Des Devel Ther 2017; 11: 163-173.

63. Qin T, Yin S, Yang J, et al. Sinomenine attenuates renal fibrosis through Nrf2-mediated inhibition of oxidative stress and TGFβ signaling. Toxicology and applied pharmacology 2016; 304: 1-8.

64. Zhang X, He H, Liang D, et al. Protective effects of Berberine on renal injury in streptozotocin (STZ)-induced diabetic mice. International journal of molecular sciences 2016; 17:

65. Wang FM, Yang YJ, Ma LL, Tian XJ, He YQ. Berberine ameliorates renal interstitial fibrosis induced by unilateral ureteral obstruction in rats. Nephrology (Carlton, Vic) 2014; 19: 542-551.

66. Cheng H, Bo Y, Shen W, et al. Leonurine ameliorates kidney fibrosis via suppressing TGF-β and NF-kappaB signaling pathway in UUO mice. International immunopharmacology 2015; 25: 406-415.

67. Wang B, Liu D, Zhu QH, et al. Rutin ameliorates kidney interstitial fibrosis in rats with obstructive nephropathy. International immunopharmacology 2016; 35: 77-84.

68. Yang J, Kan M, Wu GY. Bergenin ameliorates diabetic nephropathy in rats via suppressing renal inflammation and TGF-β1-Smad3 pathway. Immunopharmacology and immunotoxicology 2016; 38: 145-152.

69. Wang HW, Shi L, Xu YP, Qin XY, Wang QZ. Oxymatrine inhibits renal fibrosis of obstructive nephropathy by downregulating the TGF-β1-Smad3 pathway. Renal failure 2016; 38: 945-951.

70. Liu L, Wang Y, Yan R, et al. Oxymatrine inhibits renal tubular EMT induced by high glucose via upregulation of SnoN and inhibition of TGF-β1/Smad signaling pathway. PloS one 2016; 11: e0151986.

71. Lee ES, Kim HM, Kang JS, et al. Oleanolic acid and N-acetylcysteine ameliorate diabetic nephropathy through reduction of oxidative stress and endoplasmic reticulum stress in a type 2 diabetic rat model. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2016; 31: 391-400.

72. Wang DT, Huang RH, Cheng X, Zhang ZH, Yang YJ, Lin X. Tanshinone IIA attenuates renal fibrosis and inflammation via altering expression of TGF-β/Smad and NF-kappaB signaling pathway in 5/6 nephrectomized rats. International immunopharmacology 2015; 26: 4-12.

73. Zhang L, Li Z, He W, et al. Effects of Astragaloside IV against the TGF-β1-induced epithelial-to-mesenchymal transition in peritoneal mesothelial cells by promoting Smad 7 expression. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology 2015; 37: 43-54.

74. Wang L, Chi YF, Yuan ZT, et al. Astragaloside IV inhibits renal tubulointerstitial fibrosis by blocking TGF-β/Smad signaling pathway in vivo and in vitro. Experimental biology and medicine (Maywood, NJ) 2014; 239: 1310-1324.

75. Luo Q, Tian L, Di L, et al. (+/-)-Sinensilactam A, a pair of rare hybrid metabolites with Smad3 phosphorylation inhibition from Ganoderma sinensis. Organic letters 2015; 17: 1565-1568.

76. Yin J, Liao SX, He Y, et al. Dysbiosis of gut microbiota with reduced trimethylamine-N-oxide level in patients with large-artery atherosclerotic stroke or transient ischemic attack. Journal of the American Heart Association 2015; 4:

77. Chen H, Cao G, Chen DQ, et al. Metabolomics insights into activated redox signaling and lipid
metabolism dysfunction in chronic kidney disease progression. Redox Biol 2016; 10: 168–178.

78. Kansanen E, Kuosmanen SM, Leinonen H, Levonen AL. The Keap1-Nrf2 pathway: Mechanisms of activation and dysregulation in cancer. Redox Biol 2013; 1: 45-49.

79. Milkovic L, Zarkovic N, Saso L. Controversy about pharmacological modulation of Nrf2 for cancer therapy. Redox Biol 2017; 12: 727-732.

80. Chen DQ, Cao G, Chen H, et al. Gene and protein expressions and metabolomics exhibit activated redox signaling and wnt/β-catenin pathway are associated with metabolite dysfunction in patients with chronic kidney disease. Redox Biol 2017; 12: 505-521.

81. Ruiz S, Pergola PE, Zager RA, Vaziri ND. Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease. Kidney Int 2013; 83: 1029-1041.

82. Machowska A, Carrero JJ, Lindholm B, Stenvinkel P. Therapeutics targeting persistent inflammation in chronic kidney disease. Translational research : the journal of laboratory and clinical medicine 2016; 167: 204-213.

83. Zhao YY, Cheng XL, Wei F, et al. Serum metabolomics study of adenine-induced chronic renal failure in rats by ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. Biomarkers 2012; 17: 48-55.

84. Zhao YY, Cheng XL, Wei F, et al. Intrarenal metabolomic investigation of chronic kidney disease and its TGF-β1 mechanism in induced-adenine rats using UPLC Q-TOF/HSMS/MS(E). J Proteome Res 2013; 12: 2692–2703.

85. Zhao YY, Wang HL, Cheng XL, et al. Metabolomics analysis reveals the association between lipid abnormalities and oxidative stress, inflammation, fibrosis, and Nfr2 dysfunction in aristolochic acid-induced nephropathy. Sci Rep 2015; 5: 12936.

86. Trujillo J, Chirino YJ, Molina-Iijon E, Anderica-Romero AC, Tapia E, Pedraza-Chaverri J. Renoprotective effect of the antioxidant curcumin: Recent findings. Redox Biol 2013; 1: 448-456.

87. Humphreys BD. Mechanisms of Renal Fibrosis. Annual Review of Physiology 2018; 80: 309-326.

88. Meng XM, Nikolic-Paterson DJ, Lan HY. Inflammatory processes in renal fibrosis. Nat Rev Nephrol 2014; 10: 493-503.

89. Higgins DF, Kimura K, Bernhardt WM, et al. Hypoxia promotes fibrogenesis in vivo via HIF-1 stimulation of epithelial-to-mesenchymal transition. J Clin Invest 2007; 117: 3810-3820.

90. He L, Wei Q, Liu J, et al. AKI on CKD: heightened injury, suppressed repair, and the underlying mechanisms. Kidney Int 2017; 92: 1071-1083.

91. Takaori K, Yanagita M. Insights into the Mechanisms of the Acute Kidney Injury-to-Chronic Kidney Disease Continuum. Nephron 2016; 134: 172-176.

92. Theilig F, Enke AK, Scolari B, Polzin D, Bachmann S, Koesters R. Tubular deficiency of von Hippel-Lindau attenuates renal disease progression in anti-GBM glomerulonephritis. The American journal of pathology 2011; 179: 2177-2188.

93. Syn N, Wang L, Sethi G, Thiery JP, Goh BC. Exosome-mediated metastasis: from epithelial-mesenchymal transition to escape from immunosurveillance. Trends in pharmacological sciences 2016; 37: 606-617.

94. YehYC, Wei WC, Wang YK, Lin SC, Sung JM, Tang MJ. Transforming growth factor-β1 induces Smad3-dependent β1 integrin gene expression in epithelial-to-mesenchymal transition during chronic tubulointerstitial fibrosis. The American journal of pathology 2010; 177: 1743-1754.

95. Xu J, Lamouille S, Derynck R. TGF-β-induced epithelial to mesenchymal transition. Cell Res 2009; 19: 156-172.

96. Vanholder R, Schepers E, Pletinck A, Nagler EV, Glorieux G. The uremic toxicity of indoxyl sulfate and p-cresyl sulfate: a systematic review. J Am Soc Nephrol 2014; 25: 1897-1907.

97. Sun CY, Chang SC, Wu MS. Uremic toxins induce kidney fibrosis by activating intrarenal renin-angiotensin-aldosterone system associated epithelial-to-mesenchymal transition. PLoS One 2012; 7: e34026.
98. Kim SH, Yu MA, Ryu ES, Jang YH, Kang DH. Indoxyl sulfate-induced epithelial-to-mesenchymal transition and apoptosis of renal tubular cells as novel mechanisms of progression of renal disease. Laboratory investigation; a journal of technical methods and pathology 2012; 92: 488-498.

99. Bolati D, Shimizu H, Niwa T. AST-120 ameliorates epithelial-to-mesenchymal transition and interstitial fibrosis in the kidneys of chronic kidney disease rats. Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation 2012; 22: 176-180.

100. Lau WL, Savoj J, Nakata MB, Vaziri ND. Altered microbiome in chronic kidney disease: systemic effects of gut-derived uremic toxins. Clin Sci 2018; 132: 509-522.

101. Brito JS, Borges NA, Dolenga CJ, Carraro-Eduardo JC, Nakao LS, Mafra D. Is there a relationship between tryptophan dietary intake and plasma levels of indoxyl sulfate in chronic kidney disease patients on hemodialysis? Jornal brasileiro de nefrologia : 'orgao oficial de Sociedades Brasileira e Latino-Americana de Nefrologia 2016; 38: 396-402.

102. Nazzal L, Roberts J, Singh P, et al. Microbiome perturbation by oral vancomycin reduces plasma concentration of two gut-derived uremic solutes, indoxyl sulfate and p-cresyl sulfate, in end-stage renal disease. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2017; 32: 1809-1817.

103. Barreto FC, Barreto DV, Liabeuf S, et al. Serum indoxyl sulfate is associated with vascular disease and mortality in chronic kidney disease patients. Clinical journal of the American Society of Nephrology : CJASN 2009; 4: 1551-1558.

104. Miyazaki T, Ise M, Seo H, Niwa T. Indoxyl sulfate increases the gene expressions of TGF-beta 1, TIMP-1 and pro-alpha 1(I) collagen in uremic rat kidneys. Kidney Int Suppl 1997; 62: S15-22.

105. Motojima M, Hosokawa A, Yamato H, Muraki T, Yoshioka T. Uremic toxins of organic anions up-regulate PAI-1 expression by induction of NF-kappaB and free radical in proximal tubular cells. Kidney Int 2003; 63: 1671-1680.

106. Saito S, Shimizu H, Yisireyili M, Nishijima F, Enomoto A, Niwa T. Indoxyl sulfate-induced activation of (pro)renin receptor is involved in expression of TGF-beta1 and alpha-smooth muscle actin in proximal tubular cells. Endocrinology 2014; 155: 1899-1907.

107. Shimizu H, Yisireyili M, Nishijima F, Niwa T. Indoxyl sulfate enhances p53-TGF-beta1-Smad3 pathway in proximal tubular cells. American journal of nephrology 2013; 37: 97-103.

108. Galichon P, Finianos S, Hertig A. EMT-MET in renal disease: should we curb our enthusiasm? Cancer letters 2013; 341: 24-29.

109. Bolati D, Shimizu H, Higashiyama Y, Nishijima F, Niwa T. Indoxyl sulfate induces epithelial-to-mesenchymal transition in rat kidneys and human proximal tubular cells. Am J Nephrol 2011; 34: 318-323.

110. Meng X-M, Huang XR, Xiao J, et al. Disruption of Smad4 impairs TGF-β/Smad3 and Smad7 transcriptional regulation during renal inflammation and fibrosis in vivo and in vitro. Kidney international 2012; 81: 266-279.

111. Nariman-Saleh-Zam F, Bastami M, Ardalan M, Sharifi S, Hosseiniyan Khatib SM, Zununi Vahed S. Cell-free microRNA-148a is associated with renal allograft dysfunction: Implication for biomarker discovery. Journal of Cellular Biochemistry 2018; 0:

112. Ma Y, Shi J, Wang F, et al. MiR-130b increases fibrosis of HMC cells by regulating the TGF-β1 pathway in diabetic nephropathy. Journal of Cellular Biochemistry 2018; 0: