Title
New insights into TGF-β/Smad signaling in tissue fibrosis

Authors
Hu, He-He
Chen, Dan-Qian
Wang, Yan-Ni
et al.

Publication Date
2018-08-01

DOI
10.1016/j.cbi.2018.07.008

Peer reviewed

Chemico-Biological Interactions 292 (2018) 76–83

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

New insights into TGF-β/Smad signaling in tissue fibrosis

He-He Hu[a], Dan-Qian Chen[a], Yan-Ni Wang[a], Ya-Long Feng[a], Gang Cao[c], Nosratola D. Vaziri[b], Ying-Yong Zhao[a,*]

[a] Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, School of Life Science, Northwest University, No. 229 Taibai North Road, Xi'an, Shaanxi, 710069, China
[b] Division of Nephrology and Hypertension, School of Medicine, University of California Irvine, Irvine, CA, 92897, USA
[c] School of Pharmacy, Zhejiang Chinese Medical University, No. 548 Binwen Road, Hangzhou, Zhejiang, 310053, China

ARTICLE INFO

Keywords:
TGF-β
Smad
Renal fibrosis
Pulmonary fibrosis
Cardiac fibrosis
Cancer

ABSTRACT

Transforming growth factor-β1 (TGF-β1) is considered as a crucial mediator in tissue fibrosis and causes tissue scarring largely by activating its downstream small mother against decapentaplegic (Smad) signaling. Different TGF-β signalings play different roles in fibrogenesis. TGF-β1 directly activates Smad signaling which triggers pro-fibrotic gene overexpression. Excessive studies have demonstrated that dysregulation of TGF-β1/Smad pathway was an important pathogenic mechanism in tissue fibrosis. Smad2 and Smad3 are the two major downstream regulator that promote TGF-β1-mediated tissue fibrosis, while Smad7 serves as a negative feedback regulator of TGF-β1/Smad pathway thereby protects against TGF-β1-mediated fibrosis. This review presents an overview of the molecular mechanisms of TGF-β/Smad signaling pathway in renal, hepatic, pulmonary and cardiac fibrosis, followed by an in-depth discussion of their molecular mechanisms of intervention effects both *in vitro* and *in vivo*. The role of TGF-β/Smad signaling pathway in tumor or cancer is also discussed. Additionally, the current advances also highlight targeting TGF-β/Smad signaling pathway for the prevention of tissue fibrosis. The review reveals comprehensive pathophysiological mechanisms of tissue fibrosis. Particular challenges are presented and placed within the context of future applications against tissue fibrosis.

1. Introduction

Fibrosis is a wound-healing response to either acute or chronic cellular injury that is characterized by the accumulation of extracellular matrix (ECM). Excessive evidence demonstrated that fibrogenesis is associated with renin-angiotensin system, inflammation and oxidative stress, transforming growth factor β (TGF-β)/Smad signaling, Wnt/β-catenin signaling and lipid metabolism [1–10]. Among them, TGF-β/Smad signaling plays an important role in fibrosis [1,11–14]. Bona fide TGF-β is sequestered into the matrix in a latent state and must be activated before it can bind to its receptors. In recent years, more attention has been paid to TGF-β/Smad signaling pathway as an effective target of anti-fibrotic therapy [12,15,16].

TGF-β is a multi-functional mediator that regulates proliferation, differentiation, apoptosis, adhesion and migration in various cells such as macrophages, activated T and B cells, immature hematopoietic cells, neutrophils and dendritic cells [16,17]. There are three isoforms of TGF-β including transforming growth factor β1 (TGF-β1), transforming growth factor β2 (TGF-β2) and transforming growth factor β3 (TGF-β3). TGF-β1 is expressed in endothelial, hematopoietic, and connective tissue cells, and TGF-β2 is expressed in epithelial and neuronal cells, while TGF-β3 is expressed primarily in mesenchymal cells [1,17,18]. It is well-known that TGF-β1 exerts its biological effects by activating downstream mediators including Smad2 and Smad3, while is negatively regulated by Smad7 expression [12,15]. Under pathological conditions, both Smad2 and Smad3 expression are upregulated, while Smad7 is downregulated. TGF-β/Smad cascade is composed of a ternary signaling complex which been activated when TGF-β1 interacts with transforming growth factor β receptor II (TGFβRII), then TGF-βRII phosphorylates transforming growth factor β receptor I (TGFβRI), which in turn phosphorylates cytoplasmic mediators, Smad2 and/or Smad3, and a heterotrimeric complex is formed with Smad4 that translocates into the nucleus, binds a consensus sequence, and regulates gene transcription [12].

It is now well accepted that TGF-β/Smad signaling is a major pathway for fibrogenesis such as renal fibrosis, hepatic fibrosis and pulmonary fibrosis [12,16,19]. TGF-β1 is a key mediator in the development of fibrosis and inflammation. The blockade of TGF-β by neutralizing TGF-β antibodies, decorin, and antisense oligonucleotides prevents or ameliorates fibrosis. TGF-β/Smad signaling is also linked to

* Corresponding author. School of Life Science, Northwest University, No. 229 Taibai North Road, Xi'an, Shaanxi, 710069, China.
E-mail addresses: zyy@nwu.edu.cn, zhaoyybr@uci.edu (Y.-Y. Zhao).

https://doi.org/10.1016/j.cbi.2018.07.008
Received 26 May 2018; Received in revised form 1 July 2018; Accepted 9 July 2018
Available online 11 July 2018
0009-2797/

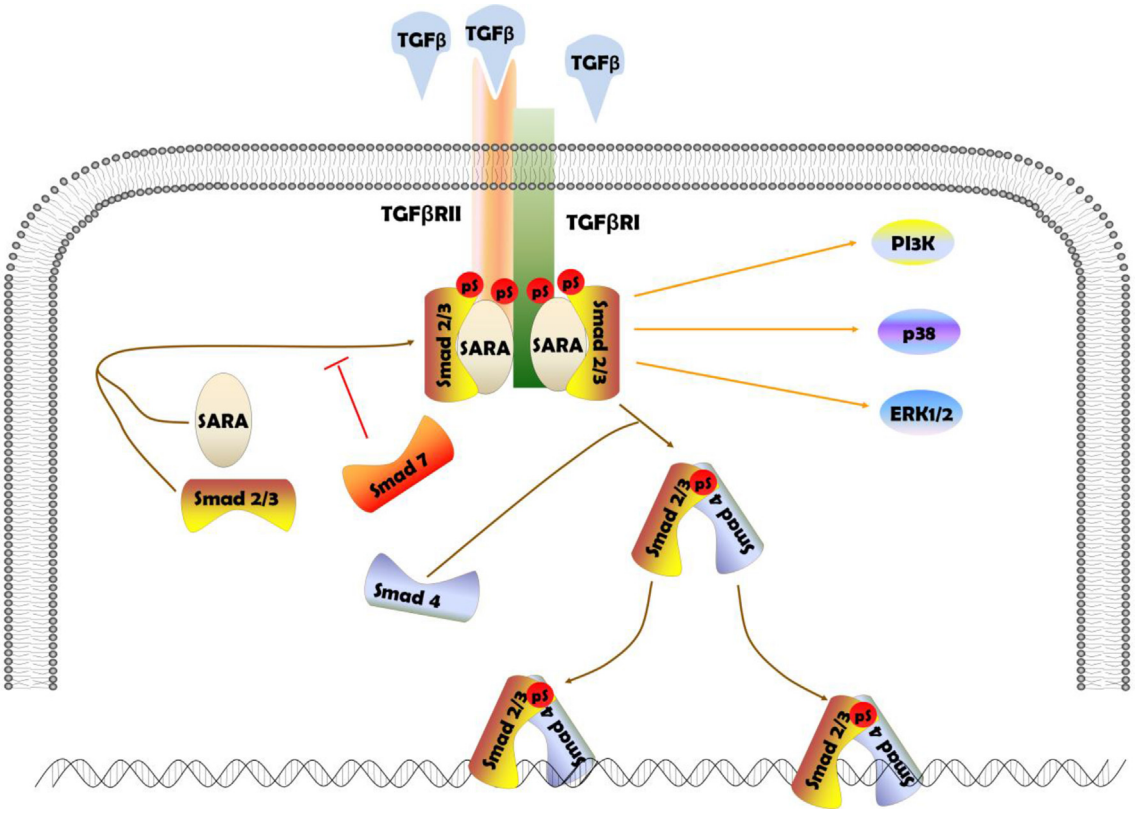

Fig. 1. Schematic diagram depicting possible mechanisms involved in the fibrogenesis of TGF-β/Smad pathway.

human carcinogenesis and other diseases [19].

This review focuses on the action mechanism of TGF-β/Smad signaling pathway and its therapeutic intervention. In addition, we also discuss the effects of other intracellular factors including tumor necrosis factor receptor-associated factor 6, TGF-β-activated kinase 1, mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), p53 and integrin on TGF-β/Smad signaling pathway [13,20–22].

2. TGF-β family

TGF-β is the prototype of a family of secreted polypeptide growth factors. It has been found that more than 40 members of this superfamily were identified since the ability to induce the ("transforming") growth of cultured fibroblasts in 1981 [23], and these members have a common dimeric structure and exhibit the presence of a cysteine knot structural motif [18]. To date up, 33 TGF-β-related genes have already been identified in mammalian genomes as the result of genome sequencing projects; these include bone morphogenic proteins (BMPs), activin/inhibin, growth and differentiation factors, nodal, and anti-Müllerian hormone. In mammals, these cytokines play very important roles, via regulating a wide array of cellular processes, such as cell growth, differentiation, migration, apoptosis, ECM production, immunity, and even embryonic development [14,17]. TGF-β family proteins control cell proliferation and differentiation. In addition, TGF-β1, TGF-β2, and TGF-β3 are induced and activated in a variety of fibrotic diseases [12,16,19,24].

2.1. TGF-β1

TGF-β1 is a multi-functional cytokine which plays a fundamental role in regulating cellular processes and ECM components including collagen, fibronectin and elastin. TGF-β1 and its two receptors TGFβRI and TGFβRII play a key role in epithelial-mesenchymal transition (EMT) and fibrogenesis [12]. The downstream molecules including smad2, smad3, smad4 and smad7 are involved in TGF-β1-induced EMT, while Smad7 blocks the smad3 expression [12].

The upregulation of TGF-β1 has been observed in lung, liver and kidney fibrosis [12,16,19]. TGF-β1 has been also identified as a key regulator of cardiac fibrosis [25], which may affect cell growth, apoptosis and differentiation, increase ECM production, maintain fibroblast viability, inhibit metalloproteinase production, and facilitate collagen degradation [26]. TGF-β1 activates Smad-dependent and -independent pathways to exhibit its biological activities [27]. Phosphorylation of Smad2 and Smad3 that forms a complex with Smad4 moves into nucleus to regulate downstream proteins. TGF-β1 can induce renal fibrosis in both canonical (Smad-based) and non-canonical (non-Smad-based) signaling pathways, which result in activation of myofibroblasts, excessive production of ECM and inhibition of ECM degradation [1]. In recent years, studies have found that TGF-β1/Smad signal transduction is a key pathway for orientation, differentiation, development and proliferation of osteoblasts. However, TGF-β1 has powerful immunosuppressive effects. Some studies demonstrated that TGF-β1 exhibited pro-inflammatory effect by upregulating the expression of chemotactic factors and pro-inflammatory factors in mesangial and inflammatory cells [28,29].

2.2. Bone morphogenic proteins

Bone morphogenic proteins (BMP) arm of the TGF-β pathway is a key regulator and BMP-7 had a protective effect in podocyte differentiation via Smad signaling [30]. Smad2 and Smad3 induce TGF-β1 activity whereas Smad1, Smad5 and Smad8 activate BMP pathway. Activation of Smad1/5/8 upregulates BMP and inhibits TGF-β1 mediated fibrotic gene expression [31]. Activation of Smad2/3 regulates TGF-β to promote fibrosis, whereas increasing BMP/Smad1/5/8 activity inhibits fibrosis (Fig. 2).

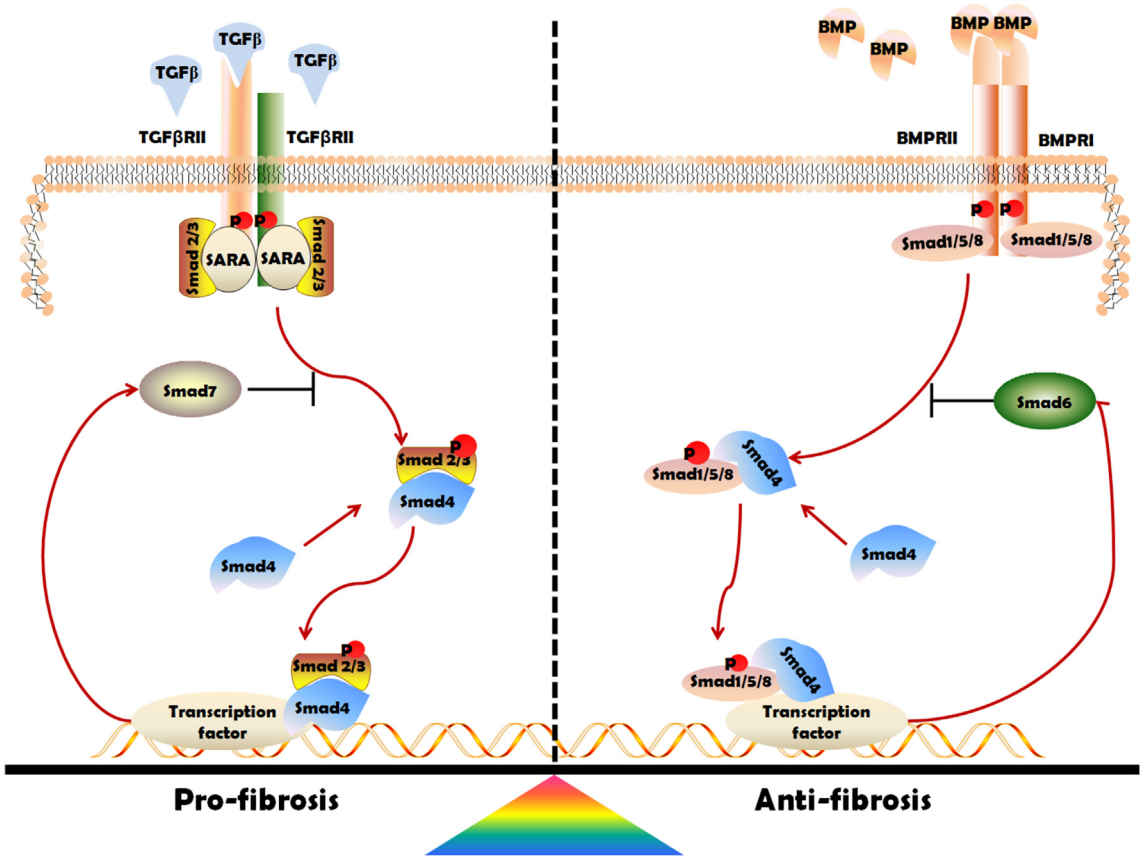

Fig. 2. The balance between TGF-β/Smad and BMP/Smad on fibrogenesis.

3. The mechanism of TGF-β/Smad signaling pathway

As shown in Fig. 1, TGF-β expresses signaling responses via both canonical (Smad-based) and non-canonical (non-Smad-based) signaling pathways [32]. TGF-β activates downstream mediators that further activates several intracellular signaling pathways to regulate various cellular functions [33]. TGF-β receptors activate Smad-based and non-Smad-based signaling pathways that substantially contribute to the TGF-β response.

TGF-β family signaling is activated by mature polypeptide then converts into a disulfide-linked dimer that acts as ligand to the cell surface receptors [34], then signaling initiates at the plasma membrane when TGF-β bind the TGF-β receptors, receptor ectodomain glycosylation and shedding. TGF-β binding induces a constitutively active type II receptor to phosphorylation of serine and/or threonine residues in the juxtamembrane GS domain of the type I receptor, which then confers conformational activation of the type I receptor kinase [35]. Furthermore, TGF-β binding to the cell surface receptor complex activates both Smad-mediated and non-Smad signaling pathways. TGF-β or activin binding to their respective receptor complexes activates Smad2 and Smad3, whereas BMP induces activation of Smad1 and Smad5 [35,36]. Upon activation by TGF-β1, Smad2 and Smad3 are phosphorylated and form a complex via Smad signaling, which is mediated by receptors that internalizes in clathrin-coated pits and is regulated by receptor, and further activates the transcription of TGF-β/Smad target genes.

Target genes of TGF-β/Smad could regulate and restore Smad complex. Among them, *AGAC* or *GTCT* are the most commonly sequence which efficiently activates TGF-β-induced transcription [18,27]. TGF-β activation also results in excessive ECM deposition including fibrillin, fibronectin and thrombospondin, which either directly or indirectly control TGF-β activation [37].

4. TGF-β/smad in normal physiological and disease processes

TGF-β acts as potent growth inhibitor for many different cells and plays a key role in the control of parenchymal apoptosis. Latent TGF-β1 plays a protective role in renal fibrosis and inflammation. Based on the current advances in understanding the pleiotropic reactions, various inhibitors of TGF-β have been developed [12,29]. TGF-β is also a potent anti-tumor agent because it strongly inhibits epithelial cell growth. However, TGF-β could also promote tumor growth because it could induce changes in transcriptional activities that reprograms epithelial cells into mesenchymal cells, thereby facilitating tumor metastasis and invasion [19].

4.1. TGF-β/smad in fibrosis

Numerous studies have clarified TGF-β pathway was closely associated with ECM gene expression and fibrogenesis [15]. Fibrosis is primarily driven by inflammatory cytokines including members of the TGF-β superfamily [1,38], various interleukins, oxidative stress and inflammation. Among them, TGF-β serves as an important and crucial mediator in fibrogenesis. TGF-β/Smad is a major signaling pathway leading to kidney disease, and mediates renal fibrosis [1].

4.1.1. TGF-β/smad signaling pathway in renal fibrosis

Renal fibrosis is main pathological features of chronic kidney disease (CKD) with high morbidity and mortality. Renal fibrosis plays a central role in the pathogenesis and progression of CKD to end stage renal disease. TGF-β1 is the central mediator of renal fibrosis and numerous studies have focused on inhibition of TGF-β1 and its downstream target genes for treatment of CKD [12]. Smad-dependent signaling pathway played a critical role in pathogenesis of CKD [1].

TGF-β/Smad signaling mediated inflammation and renal fibrosis.

TGF-β1 mediated progressive renal fibrosis by stimulating production and suppressing degradation of ECM [12]. It has been demonstrated that angiotensin II stimulated expression of TGF-β1 and its receptors [39]. Myofibroblasts were a predominant source for ECM production and myofibroblasts activation was the key step in renal fibrosis. Taken together, TGF-β1 by driving the differentiation of quiescent fibroblasts into matrix secreting myofibroblasts promoted renal fibrosis [40]. The major downstream mediators of TGF-β1 were Smad2 and Smad3. It has been demonstrated that Smad3 mainly mediated renal fibrosis, and deletion of Smad3 reduced fibrogenesis [12]. Downregulation of Smad2/3 phosphorylation and upregulation of Smad7 restored podocyte injury, and prevented renal fibrosis in kidneys by inhibiting TGF-β1 expression [41]. Smad4 was a key modulator of the TGF-β1-mediated fibrosis and inflammation by interplaying with Smad3 and Smad7 to affect their transcriptional activity in renal inflammation and fibrosis [12]. TGF-β1 was not the sole mediator of the Smad signaling which activated CKD [1,42], while many other mediators could also activated Smad2 and Smad3 in the TGF-β1-independent manner. Additionally, TGF-β/Smad pathway was regulated by ubiquitination. The components of ubiquitin-proteasome system could tightly control TGF-β/Smad signaling and renal fibrosis caused by distorting specific ubiquitin modifying enzymes [43]. Post-translational regulation of TGF-β1/Smad signaling by ubiquitination was involved in renal fibrosis and provided a novel target for treatment of CKD and renal fibrosis.

It has been reported the important role of Smad2 and Smad4 in renal fibrosis. The important findings demonstrated that Smad2 and Smad4 conditional knockout (KO) modulated renal fibrosis. The mice with constitutive deletion of Smad2 were embryonic lethal [44]. Conditional deletion of Smad2 could significantly attenuate renal fibrosis, tubular EMT-like changes and the levels of myofibroblast markers in diabetic nephropathy mice, as well as decreased Smad3 and TGF-β1 [45]. Besides, Smad2 deletion promoted fibrosis through enhanced TGF-β/Smad3 signaling, and increased autoinduction of TGF-β1. However, Smad4 in fibrosis remains unknown, and this may be attributed to the unavailability of Smad4 KO mice due to the early embryonic lethality [46]. Conditional deletion of Smad4 inhibited renal fibrosis and TGF-β1-induced collagen I expression [47].

4.1.2. TGF-β/smad signaling pathway in hepatic fibrosis

TGF-β contributed to almost all of the stages of disease progression which was regarded as a central regulator in the development and pathogenesis of liver disease. Extensive studies have demonstrated that TGF-β played a crucial role in the pathogenesis of various liver diseases, such as hepatitis and cirrhosis even hepatocellular carcinoma [15].

TGF-β1 was the most fibrogenic cytokine in liver which was derived from paracrine and autocrine sources [48]. TGF-β1 was the most abundant isoform in liver which was secreted from immune, stellate and epithelial cells. TGF-β1 triggers hepatic fibrosis mainly by mediating the activation of hematopoietic stem cells and produces excessive ECM [15,49]. An imbalance between positive and negative Smad signaling may play a vital role in the development of hepatic fibrosis. Smad3 and Smad4 were pro-fibrotic, in contrast, Smad7 were anti-fibrotic. It has been demonstrated that both Smad2 and Smad3 were strongly activated in liver fibrosis [49]. Smad3 appeared to be a key element responsible for fibrosis [19,49,50]. Smad4 interacted with Smad2/3 and participated in the transcription of downstream pro-fibrotic target genes, while Smad7 negatively regulated TGF-β/Smad signaling in two different ways. One was that Smad7 bound to TGFβRI to prevent the phosphorylation of Smad2/3, another way was that Smad7 recruited the E3 ubiquitin ligase Smad ubiquitination regulatory factors to Smad2 and TGFβRI ubiquitinated and degraded these two proteins.

4.1.3. TGF-β/smad signaling pathway in lung fibrosis

Pulmonary fibrosis included idiopathic pulmonary fibrosis (IPF) and interstitial lung fibrosis which are particularly austere lung disease [51–53]. TGF-β is the most potent profibrotic mediator to modulate lung fibrosis through recruiting and activating monocytes and fibroblasts, and the induction of ECM production [54]. Cystic fibrosis as a genetic disease was characterized by progressive lung disease. TGF-β as a master regulator of pulmonary health and disease may offer insights into new approach toward our understanding cystic fibrosis. TGF-β signaling was reported to be increased in the macrophages, airway epithelium, smooth muscle cells and fibroblasts in various pulmonary diseases [54]. The mechanism demonstrated that TGF-β negatively affected lung health in cystic fibrosis via directly downregulation of chloride transport. Several studies showed that TGF-β1 stimulation in human airway epithelial cells caused downregulated expression and function of cystic fibrosis transmembrane conductance regulator protein and an alternative chloride transport pathway [55]. TGF-β1 also promoted networks of gene expression that drive pathologic airway remodeling. Goblet cell hyperplasia and increased mucin secretion was a well feature of cystic fibrosis in lung. Inhibition of TGF-β1 signaling suppressed goblet cell hyperplasia via Smad dependent pathways in mouse models of allergen induced rhinitis and airway remodeling [56]. Recently study showed that TGF-β1 stimulation could drive airway smooth muscle shortening and hyperresponsiveness by Smad signaling [57]. Taken together, TGF-β was associated with pulmonary fibrosis in multiple animal models and in IPF and drives myofibroblast differentiation. Activation of TGF-β signaling were associated with fibrosis area and myofibroblast differentiation, furthermore, that constrictive bronchiolitis in lung biopsies was linked to myofibroblast differentiation potentially induced through TGF-β [58].

IPF as a devastating interstitial lung disease was characterized by cell injury, tissue remodeling and final lung fibrosis. TGF-β was increased in airway epithelium and fibroblasts from patients with IPF [59]. TGF-β also caused myofibroblast differentiation from fibroblasts and aberrant injury response that enhanced progressive fibrosis in IPF. In mice, inhibition of TGF-β signaling retarded pulmonary fibrosis [60]. TGF-β signaling was also involved in the pathogenesis of chronic obstructive pulmonary disease and asthma, which were characterized by airway obstruction, inflammation and remodeling [61]. TGF-β1 was increased in airway and alveolar epithelium of patients with chronic obstructive pulmonary disease and in bronchoalveolar lavage fluid obtained from asthmatic patients [54]. These studies revealed the complex and important role of TGF-β signaling in lung fibrosis. While TGF-β has been considered as a potential therapeutic target in asthma, no approved medications targeting TGF-β in asthma have advanced to the clinic [62].

4.1.4. TGF-β/smad signaling pathway in cardiac fibrosis

Chronic heart failure (CHF) is characterized by the inability of the heart to maintain a normal cardiac output without invoking maladaptive compensatory mechanisms [63]. Several studies have been explored both Smad-dependent and Smad-independent pathways contributed to cardiac fibrosis in TGF-β-induced signaling pathway [25]. In addition to being involved in canonical Smad signaling, TGF-β was also implicated in Smad-independent pathways, which also involve some members of the MAPK family. TGF-β stimulated all of the three known MAPK pathways namely, the extracellular signal-regulated kinase, Jun N-terminal kinase and p38 pathways [64]. Then, transcription factors that were the main targets for activated MAPK stimulated, and this caused the initiation of many downstream signalings. In fact, these downstream signaling pathways play crucial roles in the development of myocardial fibrosis [25]. Taken together, significant activation of TGF-β1/Smad3 and p38MAPK pathways in cardiac fibrosis can lead to myofibroblast proliferation and a marked upregulation of collagen I expression [25].

4.2. TGF-β/smad signaling pathway in tumor suppression and cancer progression

TGF-β/Smad signaling pathway was key determinants of carcinoma cell behavior and the autocrine and paracrine effects of TGF-β on tumor cells [65]. The tumor micro-environment exerted both positive and negative influences on cancer development. In brief, TGF-β/Smad signaling exhibits antiprolifertative effects in cancer [16,65]. Additionally, TGF-β1 contributes to the processes of carcinogenesis in many cancer cells which included invasion, migration, mesenchymal transition and extracellular matrix synthesis [65]. TGF-β/Smad pathway was also an important tumor suppressor in several cancers, and there was a strong correlation between malignant progression and loss of sensitivity to the antiprolifertative effects of TGF-β, which was related to reducing expression or mutational inactivation of TGF-β receptors [19,65].

Overproduction of TGF-β1 was related to breast, prostate, lung, liver, and colon cancers. TGF-β was also a key mediator of immune tolerance, and conditional deletion of β8 integrin in dendritic cells caused severe inflammatory bowel disease and autoimmunity [19,65]. In TGF-β/Smad pathway, it was demonstrated that TGF-β was widely overexpressed in many cancers and this alteration in tumors was related to poor prognosis, tumor vascularization and metastasis in contrast to the tumor suppressive effects [19,66,67].

Smad alterations played a significant role in human tumor. Accumulating evidence showed that the expression of pivotal tumor inhibitory gene was closely related to Smads. Smad4 mutation was the most common protein instability in tumors and it played a crucial role in tumor suppression [19,67]. Smad2 mapped close to Smad4, affected Smad2 phosphorylation and increased Smad2 auto-inhibition influence protein stability and suppress tumor [67,68]. Loss of Smad3 expression in tumor has been identified that have lost some TGF-β responsiveness [18,67]. Smad7 overexpression has been shown to increase the tumorigenicity in tumor cell line [69]. Taken together, Smads play a significant role in tumor.

5. Targeting TGF-β mediated Smad signaling for the prevention of tissue fibrosis

Natural products become more and more popular because it is relatively inexpensive and widely available, and has fewer adverse effects [70–72]. Natural products have been clinically used for thousands of years as an important alternative remedy for various diseases [73–75]. Compared with western medicine, natural products are a “system-to-system” treatment mode, and focus on both pathological and physiological changes. Natural products are considered to be multi-components and multi-targeted agents that exert their therapeutic functions holistically.

5.1. Targeting TGF-β1/smad signaling by isolated compounds

Natural products have been widely used as an alternative therapy against renal fibrosis [76–79]. Recent studies demonstrated that small molecular compounds from natural products could modulate TGF-β/Smad signaling pathway to treat different disease. *Poria cocos* is a well-known fungus that grows around the roots of pine trees in Asia and North America [80]. It has been frequently prescribed as one of the chief ingredients in composite prescriptions in traditional Chinese medicine (TCM). Excessive studies showed that *Poria cocos* possessed immune function, anti-tumor, anti-inflammatory, anti-fibrotic and lipid-lowing and diuretic effects [81–91]. Poricoic acid ZA is a new small compound isolated from the surface layer of *Poria cocos*, which suppressed TGFβ1/Smad pathway through inhibiting Smad2/3 phosphorylation via blocking Smad2/3-TGFβRI protein interaction [92]. Poricoic acid ZC, poricoic acid ZD and poricoic acid ZE treatment significant attenuated EMT production by inhibiting specific Smad3 phosphorylation by blocking the interaction of TGFβRI with Smad3 signaling in both TGF-β1- and AngII-treated HK-2 cells and unilateral ureteralocclusion (UUO) mice [93]. Poricoic acid ZG and poricoic acid ZH is used for treating podocyte injury and renal fibrosis [94]. Poricoic acid ZG and poricoic acid ZH were also used for renal disease by inhibiting TGF-β/Smad pathway which selectively inhibiting the phosphorylation of Smad3 via blocking the interactions of SARA with TGFβRI and Smad3 [94].

Salvia miltiorrhiza (SM) was used for treating cardiovascular and renal diseases. The previous studies showed the SM exhibited significant protective effects on CKD. Research demonstrated that its extract alleviated adenine-induced CKD via modulation of NADPH oxidase/ROS/ERK and TGF-β/Smad signaling pathways [95]. Salvianolic acid A could repress renal fibrosis and improve renal function by inhibiting TGF-β1/Smad signaling pathway [96]. Salvianolic acid B could inhibit MAPK and Smad signaling in activated hepatic stellate cells [97]. It inhibited the Smad and MAPK pathway in hepatic stellate cells stimulated with TGF-β1 [98,99].

Baicalin and baicalein isolated from *Scutellarise radix* exhibit anti-fibrotic effect *in vitro* by inhibiting the TGF-β1 pathway. Baicalein remarkably improved renal dysfunction, ameliorated kidney fibrosis and alleviated EMT and oxidative stress in hyperuricemia mice [100]. Wogonin and wogonoside effectively blocked the TGF-β1-triggered fibrotic response both *in vitro* and *in vivo* by exerting inhibitory effects on TGF-β/Smad3 signaling, indicating wogonin may be utilized as a potential anti-fibrotic effects [101]. *Scutellaria barbata* (SB) is a promising medicinal natural product. It was previously reported that the ethanol extract of SB was able to promote apoptosis, and inhibit cell proliferation and angiogenesis in human colon cancer cells, and SB could inhibit colorectal cancer cell metastasis via suppressing TGF-β/Smad signaling pathway [102]. It has been indicated that paeoniflorin treatment suppressed renal fibrosis and the production of inflammatory cytokines by altering NF-κB and TGF-β1/Smad pathway in the kidneys [103].

Alismatis rhizome (AR), as a well-known herb, exhibited lipid-lowing and renoprotective effects [104–107]. Our results indicated that AR treatment retarded renal fibrosis by downregulating inflammation and TGF-β1/Smad pathway in CKD rats [108]. Our further study demonstrated that alisol B 23-acetate treatment significantly amileriated podocytes and tubular epithelial cells by inhibiting activation of Wnt/β-catenin, oxidative stress and inflammation [109]. 25-*O*-methylalisol F is a new tetracyclic triterpenoid compound isolated from the AR. Our study found that 25-*O*-methylalisol F suppressed EMT by inhibiting Smad3 phosphorylation and promoting Smad7 expression in TGF-β/Smad-dependent pathway and it has an important effect on crosstalk between TGF-β/Smad and Wnt/β-catenin pathway in EMT process by activation of RAS [110].

Traditional Chinese medicine Shenqiwan efficiently inhibited the mRNA and protein expression of *p*-Smad2/3 by upregulating Smad7. These results suggested that Shenqiwan could retarded progressive renal fibrosis, possibly by inhibiting TGF-β1/Smad signaling pathway [111]. The protective effects of HuangQi decoction for kidney damage in UUO mice was mediated by downregulating the TGF-β/Smad signaling. Similarly, renal fibrosis was attenuated or suppressed through TGF-β/Smad pathway by various natural products, such as Tanshinone HA [112], sinomenine [113], oxymatrine [114], bergenin [115], Wulingsan [116] and You-gui Pill [117].

5.2. Targeting TGF-β1/smad signaling by extracts and compound prescriptions

Polysaccharide (ESP-B4) from *Ephedra sinica* Stapf against airway and pulmonary inflammation revealed that ESP-B4 could downregulate the production of TGF-β1, *p*-Smad2 and *p*-Smad3, upregulate the expression of Smad7, which indicated that ESP-B4 reduced airway and pulmonary inflammation by regulating the TGF-β1/Smad2 pathway [118]. Shen-mai-kai-fei-san (Shenks) has been shown to be effective in

the treatment of pulmonary fibrosis [119]. *In vivo* and *in vitro* studies demonstrated that Shenks inhibited fibrosis by blocking TGF-β pathway.

It has been reported that phenylethanol glycosides from *Cistanche tubulosa* could block the conduction of the signaling pathways in TGF-β1/Smad, and inhibit the activation of hepatic stellate cells, suggesting that it may be a potential agents for the treatment of liver fibrosis [120]. Liuweiwuling tablets were supported by the inhibition of the TGF-β/Smad-mediated synthesis of collagen I in hepatic fibrosis [121]. Buyanghuanwu decoction is a representative prescription for the treatment of qi-deficiency and blood-stasissyndrome and Buyanghuanwu decoction exerted a cardioprotective effect against pressure overload induced cardiac remodeling via inactivation of TGF-β/Smad signaling triggered fibrosis [122].

6. Conclusion

In summary, the present review provides a systemic review for TGF-β/Smad signaling pathway in tissue fibrosis. The current advances in research into TGF-β/Smad pathway improve our understanding of the molecular mechanisms of fibrogenesis in renal, hepatic, pulmonary and cardiac fibrosis. Activation of Smad and non-Smad signaling contributes to the pathological roles of TGF-β signaling. Smads exert their functions through post-translational modifications such as phosphorylation, acetylation, sumoylation, ubiquitination and protein-protein interactions. Therefore, understanding of the specific function of Smad-dependent signaling pathways and their crosstalk in tissue fibrosis would help the development of specific and effective therapeutic strategies for specific tissue fibrosis. Particular challenges are presented and placed within the context of future applications against fibrosis.

Conflicts of interest

All the authors declared no competing interests.

Acknowledgment

This study was supported by the National Natural Science Foundation of China (Nos. 81673578, 81603271).

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.cbi.2018.07.008.

References

[1] X.M. Meng, D.J. Nikolic-Paterson, H.Y. Lan, TGF-β: the master regulator of fibrosis, Nat. Rev. Nephrol. 12 (2016) 325–338.
[2] L. Chen, W. Su, H. Chen, D.Q. Chen, M. Wang, Y. Guo, Y.Y. Zhao, Proteomics for biomarker identification and clinical application in kidney disease, Adv. Clin. Chem. 85 (2018) 91–113.
[3] Y.Y. Zhao, Metabolomics in chronic kidney disease, Clin. Chim. Acta 422 (2013) 59–69.
[4] X.M. Meng, P.M.K. Tang, J. Li, H.Y. Lan, TGF-β/Smad signaling in renal fibrosis, Front. Physiol. 6 (2015) 82.
[5] Y.Y. Zhao, R.C. Lin, Metabolomics in nephrotoxicity, Adv. Clin. Chem. 65 (2014) 69–89.
[6] D.Q. Chen, G. Cao, H. Chen, D. Liu, W. Su, X.Y. Yu, N.D. Vaziri, X.H. Liu, X. Bai, L. Zhang, Y.Y. Zhao, 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. 12 (2017) 505–521.
[7] Y.Y. Zhao, N.D. Vaziri, R.C. Lin, Lipidomics: new insight into kidney disease, Adv. Clin. Chem. 68 (2015) 153–175.
[8] Y.Y. Zhao, H.L. Wang, X.L. Cheng, F. Wei, X. Bai, R.C. Lin, N.D. Vaziri, Metabolomics analysis reveals the association between lipid abnormalities and oxidative stress, inflammation, fibrosis, and Nrf2 dysfunction in aristolochic acid-induced nephropathy, Sci. Rep. 5 (2015) 12936.
[9] Z.H. Zhang, J.R. Mao, H. Chen, W. Su, Y. Zhang, L. Zhang, D.Q. Chen, Y.Y. Zhao, N.D. Vaziri, Removal of uremic retention products by hemodialysis is coupled with indiscriminate loss of vital metabolites, Clin. Biochem. 50 (2017) 1078–1086.
[10] D.Q. Chen, H. Chen, L. Chen, N.D. Vaziri, M. Wang, X.R. Li, Y.Y. Zhao, The link between phenotype and fatty acid metabolism in advanced chronic kidney disease, Nephrol. Dial, Transplant 32 (2017) 1154–1166.
[11] A. Loboda, M. Sobczak, A. Jozkowicz, J. Dulak, TGF-β1/Smads and miR-21 in renal fibrosis and inflammation, Mediators inflamm 2016 (2016) 8319283.
[12] L. Chen, T. Yang, D.W. Lu, H. Zhao, Y.L. Feng, H. Chen, D.Q. Chen, N.D. Vaziri, Y.Y. Zhao, Central role of dysregulation of TGF-β/Smad in CKD progression and potential targets of its treatment, Biomed. Pharmacother. 101 (2018) 670–681.
[13] S.P. Higgins, Y. Tang, C.E. Higgins, B. Mian, W. Zhang, R.P. Czekay, R. Samarakoon, D.J. Conti, P.J. Higgins, TGF-β1/p53 signaling in renal fibrogenesis, Cell. Signal. 43 (2018) 1–10.
[14] F. Verrecchia, F. Redini, Transforming growth factor-β signaling plays a pivotal role in the interplay between osteosarcoma cells and their microenvironment, Front. Oncol 8 (2018) 133.
[15] K.L. Walton, K.E. Johnson, C.A. Harrison, Targeting TGF-β mediated SMAD signaling for the prevention of fibrosis, Front. Pharmacol. 8 (2017) 461.
[16] P.O. Eser, P.A. Janne, TGFβ pathway inhibition in the treatment of non-small cell lung cancer, Pharmacol. Ther. 184 (2018) 112–130.
[17] J. Nickel, P. Ten Dijke, T.D. Mueller, TGF-β family co-receptor function and signaling, Acta Biochim. Biophys. Sin. 50 (2018) 12–36.
[18] A. Chaikuad, A.N. Bullock, Structural basis of intracellular TGF-β signaling: receptors and Smads, Cold Spring Harb. Perspect. Biol. 8 (2016) a022111.
[19] L.H. Katz, M. Likhter, W. Jogunoori, M. Belkin, K. Ohshiro, L. Mishra, TGF-β signaling in liver and gastrointestinal cancers, Canc. Lett. 379 (2016) 166–172.
[20] M. Landstrom, The TAK1-TRAF6 signalling pathway, Int, J. Biochem. Cell Biol. 42 (2010) 585–589.
[21] C. Margadant, A. Sonnenberg, Integrin-TGF-β crosstalk in fibrosis, cancer and wound healing, EMBO Rep. 11 (2010) 97–105.
[22] J. Abrigo, F. Campos, F. Simon, C. Riedel, D. Cabrera, C. Vilos, C. Cabello-Verrugio, TGF-β requires the activation of canonical and non-canonical signalling pathways to induce skeletal muscle atrophy, Biol. Chem. 399 (2018) 253–264.
[23] A.B. Roberts, M.A. Anzano, L.C. Lamb, J.M. Smith, M.B. Sporn, New class of transforming growth factors potentiated by epidermal growth factor: isolation from non-neoplastic tissues, Proc. Natl. Acad. Sci. U. S. A 78 (1981) 5339–5343.
[24] Y.Y. Zhao, X.L. Cheng, F. Wei, X. Bai, X.J. Tan, R.C. Lin, Q. Mei, 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. 12 (2013) 2692–2703.
[25] Y. Yue, K. Meng, Y. Pu, X. Zhang, Transforming growth factor β (TGF-β) mediates cardiac fibrosis and induces diabetic cardiomyopathy, Diabetes Res. Clin. Pract. 133 (2017) 124–130.
[26] A. Biernacka, M. Dobaczewski, N.G. Frangogiannis, TGF-β signaling in fibrosis, Growth Factors 29 (2011) 196–202.
[27] M.J. Macias, P. Martin-Malpartida, J. Massague, Structural determinants of Smad function in TGF-β signaling, Trends Biochem. Sci. 40 (2015) 296–308.
[28] X.M. Meng, D.J. Nikolic-Paterson, H.Y. Lan, Inflammatory processes in renal fibrosis, Nat. Rev. Nephrol. 10 (2014) 493–503.
[29] H.Y. Lan, Diverse roles of TGF-β/Smads in renal fibrosis and inflammation, Int. J. Biol. Sci. 7 (2011) 1056–1067.
[30] S. Yamada, J. Nakamura, M. Asada, M. Takase, T. Matsusaka, T. Iguchi, R. Yamada, M. Tanaka, A.Y. Higashi, T. Okuda, N. Asada, A. Fukatsu, H. Kawachi, D. Graf, E. Muso, T. Kita, T. Kimura, I. Pastan, A.N. Economides, M. Yanagita, Twisted gastrulation, a BMP antagonist, exacerbates podocyte injury, PLoS One 9 (2014) e89135.
[31] J.M. Munoz-Felix, M. Gonzalez-Nunez, C. Martinez-Salgado, J.M. Lopez-Novoa, TGF-β/BMP proteins as therapeutic targets in renal fibrosis. Where have we arrived after 25 years of trials and tribulations? Pharmacol. Ther. 156 (2015) 44–58.
[32] Y. Mu, S.K. Gudey, M. Landstrom, Non-Smad signaling pathways, Cell Tissue Res. 347 (2012) 11–20.
[33] Y.E. Zhang, Non-Smad signaling pathways of the TGF-β family, Cold Spring Harb. Perspect. Biol. 9 (2017) a022129.
[34] M. Morikawa, R. Derynck, K. Miyazono, TGF-β and the TGF-β family: context-dependent roles in cell and tissue physiology, Cold Spring Harb. Perspect. Biol. 8 (2016) a021873.
[35] C.H. Heldin, A. Moustakas, Signaling receptors for TGF-β family members, Cold Spring Harb. Perspect. Biol. 8 (2016) a022053.
[36] C.S. Hill, Transcriptional control by the SMADs, Cold Spring Harb. Perspect. Biol. 8 (2016) a022079.
[37] I.B. Robertson, D.B. Rifkin, Regulation of the bioavailability of TGF-β and TGF-β-related proteins, Cold Spring Harb. Perspect. Biol. 8 (2016) a021907.
[38] A. Sureshbabu, S.A. Muhsin, M.E. Choi, TGF-β signaling in the kidney: profibrotic and protective effects, Am. J. Physiol. Ren. Physiol. 310 (2016) F596–F606.
[39] Z. Liu, X.R. Huang, H.Y. Chen, E. Fung, J. Liu, H.Y. Lan, Deletion of angiotensin-converting enzyme-2 promotes hypertensive nephropathy by targeting Smad7 for ubiquitin degradation, Hypertension 70 (2017) 822–830.
[40] Y.B. Sun, X. Qu, G. Caruana, J. Li, The origin of renal fibroblasts/myofibroblasts and the signals that trigger fibrosis, Differentiation 92 (2016) 102–107.
[41] J.W. Leeuwis, T.Q. Nguyen, A. Dendooven, R.J. Kok, R. Goldschmeding, Targeting podocyte-associated diseases, Adv. Drug Deliv. Rev. 62 (2010) 1325–1336.
[42] H.Y. Lan, Transforming growth factor-β/Smad signalling in diabetic nephropathy, Clin. Exp. Pharmacol. Physiol. 39 (2012) 731–738.
[43] P. Xu, J. Liu, R. Derynck, Post-translational regulation of TGF-β receptor and Smad signaling, FEBS Lett. 586 (2012) 1871–1884.
[44] M. Nomura, E. Li, Smad2 role in mesoderm formation, left-right patterning and craniofacial development, Nature 393 (1998) 786–790.

[45] I. Loeffler, M. Liebisch, S. Allert, E. Kunisch, R.W. Kinne, G. Wolf, FSP1-specific SMAD2 knockout in renal tubular, endothelial, and interstitial cells reduces fibrosis and epithelial-to-mesenchymal transition in murine STZ-induced diabetic nephropathy, Cell Tissue Res. 372 (2018) 115–133.

[46] C. Sirard, J.L. de la Pompa, A. Elia, A. Itie, C. Mirtsos, A. Cheung, S. Hahn, A. Wakeham, L. Schwartz, S.E. Kern, J. Rossant, T.W. Mak, The tumor suppressor gene Smad4/Dpc4 is required for gastrulation and later for anterior development of the mouse embryo, Genes Dev. 12 (1998) 107–119.

[47] X.M. Meng, X.R. Huang, J. Xiao, A.C.K. Chung, W. Qin, H.Y. Chen, H.Y. Lan, Disruption of Smad4 impairs TGF-β/Smad3 and Smad7 transcriptional regulation during renal inflammation and fibrosis *in vivo* and *in vitro*, Kidney Int. 81 (2012) 266–279.

[48] R.M. Liu, L.P. Desai, Reciprocal regulation of TGF-β and reactive oxygen species: a perverse cycle for fibrosis, Redox Biol. 6 (2015) 565–577.

[49] K. Yu, Q. Li, G. Shi, N. Li, Involvement of epithelial-mesenchymal transition in liver fibrosis, Saudi J. Gastroenterol. 24 (2018) 5–11.

[50] E. Hernandez-Aquino, P. Muriel, Beneficial effects of naringenin in liver diseases: molecular mechanisms, World J. Gastroenterol. 24 (2018) 1679–1707.

[51] P.J. Wolters, H.R. Collard, K.D. Jones, Pathogenesis of idiopathic pulmonary fibrosis, Ann. Rev. Pathol 9 (2014) 157–179.

[52] L. Yan, F. Song, H. Li, Y. Li, J. Li, Q.Y. He, D. Zhang, F. Wang, M. Zhang, H. Zhao, T. Feng, Y.Y. Zhao, S.W. Wang, Submicron emulsion of cinnamaldehyde ameliorates bleomycin-induced idiopathic pulmonary fibrosis via inhibition of inflammation, oxidative stress and epithelial-mesenchymal transition, Biomed. Pharmacother. 102 (2018) 765–771.

[53] D.J. Lederer, F.J. Martinez, Idiopathic pulmonary fibrosis, N. Engl. J. Med. 378 (2018) 1811–1823.

[54] E.L. Kramer, J.P. Clancy, TGFβ as a therapeutic target in cystic fibrosis, Expert Opin. Ther. Targets 22 (2018) 177–189.

[55] H. Sun, W.T. Harris, S. Kortyka, K. Kotha, A.J. Ostmann, A. Rezayat, A. Sridharan, Y. Sanders, A.P. Naren, J.P. Clancy, Tgf-β downregulation of distinct chloride channels in cystic fibrosis-affected epithelia, PLoS One 9 (2014) e106842.

[56] Y. Ouyang, M. Miyata, K. Hatsushika, Y. Ohnuma, R. Katoh, H. Ogawa, K. Okumura, K. Masuyama, A. Nakao, TGF-β signaling may play a role in the development of goblet cell hyperplasia in a mouse model of allergic rhinitis, Allergol. Int. 59 (2010) 313–319.

[57] C.A. Ojiaku, G. Cao, W. Zhu, E.J. Yoo, M. Shumyatcher, B.E. Himes, S.S. An, R.A. Panettieri Jr., TGF-β1 evokes human airway smooth muscle cell shortening and hyperresponsiveness via Smad3, Am. J. Respir. Cell Mol. Biol. 58 (2018) 575–584.

[58] W.T. Harris, J.T. Boyd, G.L. McPhail, A.S. Brody, R.D. Szczesniak, L.L. Korbee, M.L. Baker, J.P. Clancy, Constrictive bronchiolitis in cystic fibrosis adolescents with refractory pulmonary decline, Ann. Am. Thorac. Soc. 13 (2016) 2174–2183.

[59] Y. Xu, T. Mizuno, A. Sridharan, Y. Du, M. Guo, J. Tang, K.A. Wikenheiser-Brokamp, A.T. Perl, V.A. Funari, J.J. Gokey, B.R. Stripp, J.A. Whitsett, Single-cell RNA sequencing identifies diverse roles of epithelial cells in idiopathic pulmonary fibrosis, JCI insight 1 (2016) e90558.

[60] M. Li, M.S. Krishnaveni, C. Li, B. Zhou, Y. Xing, A. Banfalvi, A. Li, V. Lombardi, O. Akbari, Z. Borok, P. Minoo, Epithelium-specific deletion of TGF-β receptor type II protects mice from bleomycin-induced pulmonary fibrosis, J. Clin. Invest. 121 (2011) 277–287.

[61] H. Kang, Role of MicroRNAs in TGF-β signaling pathway-mediated pulmonary fibrosis, Int. J. Mol. Sci. 18 (2017).

[62] M. Al-Alawi, T. Hassan, S.H. Chotirmall, Transforming growth factor β and severe asthma: a perfect storm, Respir. Med. 108 (2014) 1409–1423.

[63] P.A. Heidenreich, J.G. Trogdon, O.A. Khavjou, J. Butler, K. Dracup, M.D. Ezekowitz, E.A. Finkelstein, Y. Hong, S.C. Johnston, A. Khera, D.M. Lloyd-Jones, S.A. Nelson, G. Nichol, D. Orenstein, P.W.F. Wilson, Y.J. WooC. Amer Heart Assoc Advocacy, C. Stroke, I. Council Cardiovasc Radiology, C. Council Clinical, P. Council Epidemiology, T. Council Arteriosclerosis, C. Council Cardiopulmonary Critical, N. Council Cardiovasc, D. Council Kidney Cardiovasc, A. Council Cardiovasc Surgery, Q. Interdisciplinary Council, Forecasting the future of cardiovascular disease in the United States a policy statement from the american heart association, Circulation 123 (2011) 933–944.

[64] Z.L. Li, Y. Shi, Y. Ding, Y. Ran, G. Le, Dietary oxidized tyrosine (O-Tyr) stimulates TGF-β1-induced extracellular matrix production via the JNK/p38 signaling pathway in rat kidneys, Amino Acids 49 (2017) 241–260.

[65] B. Jung, J.J. Staudacher, D. Beauchamp, Transforming growth factor β superfamily dignaling in development of colorectal cancer, Gastroenterology 152 (2017) 36–52.

[66] P. Chandrasinghe, B. Cereser, M. Moorghen, I. Al Bakir, N. Tabassum, A. Hart, J. Stebbing, J. Warusavitarne, Role of SMAD proteins in colitis-associated cancer: from known to the unknown, Oncogene 37 (2018) 1–7.

[67] S. Shany, I. Sigal-Batikoff, S. Lamprecht, Vitamin D and myofibroblasts in fibrosis and cancer: at cross-purposes with TGF-β/SMAD signaling, Anticancer Res. 36 (2016) 6225–6234.

[68] M. Zhao, L. Mishra, C.X. Deng, The role of TGF-β/SMAD4 signaling in cancer, Int. J. Biol. Sci. 14 (2018) 111–123.

[69] L. Luo, N. Li, N. Lv, D. Huang, SMAD7: a timer of tumor progression targeting TGF-β signaling, Tumour Biol. 35 (2014) 8379–8385.

[70] Z.H. Zhang, F. Wei, N.D. Vaziri, X.L. Cheng, X. Bai, R.C. Lin, Y.Y. Zhao, Metabolomics insights into chronic kidney disease and modulatory effect of rhubarb against tubulointerstitial fibrosis, Sci. Rep. 5 (2015) 14472.

[71] M. Wang, L. Chen, D. Liu, H. Chen, D.D. Tang, Y.Y. Zhao, Metabolomics highlights pharmacological bioactivity and biochemical mechanism of traditional Chinese medicine, Chem. Biol. Interact. 273 (2017) 133–141.

[72] Y.Y. Zhao, X.L. Cheng, J.H. Cui, X.R. Yan, F. Wei, X. Bai, R.C. Lin, Effect of ergosta-4,6,8(14),22-tetraen-3-one (ergone) on adenine-induced chronic renal failure rat: a serum metabonomic study based on ultra performance liquid chromatography/high-sensitivity mass spectrometry coupled with MassLynx i-FIT algorithm, Clin. Chim. Acta 413 (2012) 1438–1445.

[73] Y.Y. Zhao, Traditional uses, phytochemistry, pharmacology, pharmacokinetics and quality control of *Polyporus umbellatus* (Pers.) Fries: a review, J. Ethnopharmacol. 149 (2013) 35–48.

[74] H. Chen, T. Tian, H. Miao, Y.Y. Zhao, Traditional uses, fermentation, phytochemistry and pharmacology of *Phellinus linteus*: a review, Fitoterapia 113 (2016) 6–26.

[75] Z.H. Zhang, N.D. Vaziri, F. Wei, X.L. Cheng, X. Bai, Y.Y. Zhao, An integrated lipidomics and metabolomics reveal nephroprotective effect and biochemical mechanism of *Rheum officinale* in chronic renal failure, Sci. Rep. 6 (2016) 22151.

[76] Y.Y. Zhao, L. Zhang, J.R. Mao, X.H. Cheng, L. R.C, Y. Zhang, W.J. Sun, Ergosta-4,6,8(14),22-tetraen-3-one isolated from *Polyporus umbellatus* prevents early renal injury in aristolochic acid-induced nephropathy rats, J. Pharm. Pharmacol. 63 (2011) 1581–1586.

[77] Y.Y. Zhao, H. Chen, T. Tian, D.Q. Chen, X. Bai, F. Wei, A pharmaco-metabonomic study on chronic kidney disease and therapeutic effect of ergone by UPLC-QTOF/HDMS, PLoS One 23 (2014) e115467.

[78] Y.Y. Zhao, L. Zhang, F.Y. Long, X.L. Cheng, X. Bai, F. Wei, R.C. Lin, UPLC-Q-TOF/HSMS/MS^E-based metabonomics 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. 201 (2013) 31–38.

[79] H. Chen, G. Cao, D.Q. Chen, M. Wang, N.D. Vaziri, Z.H. Zhang, J.R. Mao, X. Bai, Y.Y. Zhao, Metabolomics insights into activated redox signaling and lipid metabolism dysfunction in chronic kidney disease progression, Redox Biol. 10 (2016) 168–178.

[80] Y.Z. Wang, J. Zhang, Y.L. Zhao, T. Li, T. Shen, J.Q. Li, W.Y. Li, H.G. Liu, Mycology, cultivation, traditional uses, phytochemistry and pharmacology of *Wolfiporia cocos* (Schwein.) Ryvarden et Gilb.: a review, J. Ethnopharmacol. 147 (2013) 265–276.

[81] J.L. Rios, Chemical constituents and pharmacological properties of *Poria cocos*, Planta Med. 77 (2011) 681–691.

[82] S.J. Yu, J. Tseng, Fu-Ling, a Chinese herbal drug, modulates cytokine secretion by human peripheral blood monocytes, Int. J. Immunopharm. 18 (1996) 37–44.

[83] G.W. Zhang, H.Y. Liu, Q.M. Xia, J.Q. Li, H. Lu, Q.H. Zhang, Z.F. Yao, Anti-rejection effect of ethanol extract of *Poria cocos* wolf in rats after cardiac allograft implantation, Chin. Med. J. 117 (2004) 932–935.

[84] M. Zhang, L.C.M. Chiu, P.C.K. Cheung, V.E.C. Ooi, Growth-inhibitory effects of a β-glucan from the mycelium of *Poria cocos* on human breast carcinoma MCF-7 cells: cell-cycle arrest and apoptosis induction, Oncol. Rep. 15 (2006) 637–643.

[85] Y.Y. Zhao, Y.L. Feng, X. Bai, X.J. Tan, R.C. Lin, Q. Mei, Ultra performance liquid chromatography-based metabonomic study of therapeutic effect of the surface layer of *Poria cocos* on adenine-induced chronic kidney disease provides new insight into anti-fibrosis mechanism, PLoS One 8 (2013) e59617.

[86] Y.Y. Zhao, H.T. Li, Y.I. Feng, X. Bai, R.C. Lin, Urinary metabonomic study of the surface layer of *Poria cocos* as an effective treatment for chronic renal injury in rats, J. Ethnopharmacol. 148 (2013) 403–410.

[87] Y.Y. Zhao, P. Lei, D.Q. Chen, Y.L. Feng, X. Bai, Renal metabolic profiling of early renal injury and renoprotective effects of *Poria cocos* epidermis using UPLC Q-TOF/HSMS/MS^E, J. Pharmaceut. Biomed. Anal. 81–82 (2013) 202–209.

[88] H. Miao, Y.H. Zhao, N.D. Vaziri, D.D. Tang, H. Chen, H. Chen, M. Khazaeli, M. Tarbiat-Boldaji, L. Hatami, Y.Y. Zhao, Lipidomics biomarkers of diet-induced hyperlipidemia and its treatment with *Poria cocos*, J. Agric. Food Chem. 64 (2016) 969–979.

[89] Y.L. Feng, P. Lei, T. Tian, L. Yin, D.Q. Chen, H. Chen, Q. Mei, Y.Y. Zhao, R.C. Lin, Diuretic activity of some fractions of the epidermis of *Poria cocos*, J. Ethnopharmacol. 150 (2013) 1114–1118.

[90] Y.Y. Zhao, Y.L. Feng, X. Du, Z.H. Xi, X.L. Cheng, F. Wei, Diuretic activity of the ethanol and aqueous extracts of the surface layer of *Poria cocos* in rat, J. Ethnopharmacol. 144 (2012) 775–778.

[91] H. Miao, M.H. Li, X. Zhang, S.J. Yuan, C.C. Ho, Y.Y. Zhao, The antihyperlipidemic effect of Fu-Ling-Pi is associated with abnormal fatty acid metabolism as assessed by UPLC-HDMS-based lipidomics, RSC Adv. 5 (2015) 64208–64219.

[92] M. Wang, D.Q. Chen, M.C. Wang, H. Chen, L. Chen, D. Liu, H. Zhao, Y.Y. Zhao, Poricoic acid ZA, a novel RAS inhibitor, attenuates tubulo-interstitial fibrosis and podocyte injury by inhibiting TGF-β/Smad signaling pathway, Phytomedicine 36 (2017) 243–253.

[93] M. Wang, D.Q. Chen, L. Chen, G. Cao, H. Zhao, D. Liu, N.D. Vaziri, Y. Guo, Y.Y. Zhao, Novel inhibitors of the cellular renin-angiotensin system components, poricoic acids, target Smad3 phosphorylation and Wnt/β-catenin pathway against renal fibrosis, Br. J. Pharmacol. 175 (2018) 2689–2708.

[94] M. Wang, D.Q. Chen, L. Chen, H. Zhao, D. Liu, Z.H. Zhang, N.D. Vaziri, Y. Guo, Y.Y. Zhao, G. Cao, Novel RAS inhibitors poricoic acid ZG and poricoic acid ZH attenuate renal fibrosis via Wnt/β-catenin pathway and targeted phosphorylation of smad3 signaling, J. Agric. Food Chem. 66 (2018) 1828–1842.

[95] H.D. Cai, S.L. Su, Y.H. Li, H.T. Zeng, Z.H. Zhu, J.M. Guo, Y. Zhu, S. Guo, L. Yu, D.W. Qian, Y.P. Tang, J.N. Duan, Protective effects of *Salvia miltiorrhiza* on adenine-induced chronic renal failure by regulating the metabolic profiling and modulating the NADPH oxidase/ROS/ERK and TGF-β/Smad signaling pathways, J. Ethnopharmacol. 212 (2018) 153–165.

[96] Z. Ma, Y. Tang, L. Zhong, K. Yu, L. He, Anti-fibrosis and relative mechanism of salvianolic acid A on rat model with renal fibrosis, Int. J. Clin. Exp. Med. 9 (2016) 12713–12720.

[97] Z. Lv, L. Xu, Salvianolic Acid B inhibits ERK and p38 MAPK signaling in TGF-β1-

stimulated human hepatic stellate cell line (LX-2) via distinct pathways, Evid. Based Complement. Alternat. Med. 2012 (2012) 960128.
[98] P. Liu, Y.Y. Hu, C. Liu, D.Y. Zhu, H.M. Xue, Z.Q. Xu, L.M. Xu, C.H. Liu, H.T. Gu, Z.Q. Zhang, Clinical observation of salvianolic acid B in treatment of liver fibrosis in chronic hepatitis B, World J. Gastroenterol. 8 (2002) 679–685.
[99] C. Liu, P. Liu, Y. Hu, D. Zhu, Effects of salvianolic acid-B on TGF-β1 stimulated hepatic stellate cell activation and its intracellular signaling, Natl. Med. J. China (Peking) 82 (2002) 1267–1272.
[100] X. Meng, Z. Mao, X. Li, D. Zhong, M. Li, Y. Jia, J. Wei, B. Yang, H. Zhou, Baicalein decreases uric acid and prevents hyperuricemic nephropathy in mice, Oncotarget 8 (2017) 40305–40317.
[101] X.M. Meng, G.L. Ren, L. Gao, H.D. Li, W.F. Wu, X.F. Li, T. Xu, X.F. Wang, T.T. Ma, Z. Li, C. Huang, Y. Huang, L. Zhang, X.W. Lv, J. Li, Anti-fibrotic effect of wogonin in renal tubular epithelial cells via Smad3-dependent mechanisms, Eur. J. Pharmacol. 789 (2016) 134–143.
[102] Y. Jin, W. Chen, H. Yang, Z. Yan, Z. Lai, J. Feng, J. Peng, J. Lin, Scutellaria barbata D. Don inhibits migration and invasion of colorectal cancer cells via suppression of PI3K/AKT and TGF-β/Smad signaling pathways, Exp. Ther. Med. 14 (2017) 5527–5534.
[103] X. Wang, H. Tian, J. Wang, S. Liu, B. Wang, T. Luo, Paeoniflorin inhibits TGF-β 1-Smad2/3 and NF-κB signaling pathways in high fat diet-induced kidney injury and inflammatory responses, Int. J. Clin. Exp. Med. 11 (2018) 694–703.
[104] T. Tian, H. Chen, Y.Y. Zhao, Traditional uses, phytochemistry, pharmacology, toxicology and quality control of *Alisma orientale* (Sam.) Juzep: a review, J. Ethnopharmacol. 158 (2014) 373–387.
[105] H. Miao, L. Zhang, D.Q. Chen, H. Chen, Y.Y. Zhao, S.C. Ma, Urinary biomarker and treatment mechanism of *Rhizoma Alismatis* on hyperlipidemia, Biomed. Chromatogr. 31 (2017) e3829.
[106] D.Q. Chen, Y.L. Feng, T. Tian, H. Chen, L. Yin, Y.Y. Zhao, R.C. Lin, Diuretic and anti-diuretic activities of fractions of *Alismatis rhizoma*, J. Ethnopharmacol. 157 (2014) 114–118.
[107] Y.L. Feng, H. Chen, T. Tian, D.Q. Chen, Y.Y. Zhao, R.C. Lin, Diuretic and anti-diuretic activities of the ethanol and aqueous extracts of *Alismatis rhizoma*, J. Ethnopharmacol. 154 (2014) 386–390.
[108] F. Dou, H. Miao, J.W. Wang, L. Chen, M. Wang, H. Chen, A.D. Wen, Z.Y. Y, An integrated lipidomics and phenotype study reveals protective effect and biochemical mechanism of traditionally used *Alisma orientale* Juzepzuk in chronic renal disease, Front. Pharmacol. 9 (2018) 53.
[109] L. Chen, D.Q. Chen, M. Wang, D. Liu, H. Chen, F. Dou, N.D. Vaziri, Y.Y. Zhao, Role of RAS/Wnt/β-catenin axis activation in the pathogenesis of podocyte injury and tubulo-interstitial nephropathy, Chem. Biol. Interact. 273 (2017) 56–72.
[110] H. Chen, T. Yang, M.C. Wang, D.Q. Chen, Y. Yang, Y.Y. Zhao, 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 42 (2018) 207–218.
[111] H. Chen, Y. Xu, Y. Yang, X. Zhou, S. Dai, C. Li, Shenqiwan ameliorates renal fibrosis in rats by inhibiting TGF-β 1/Smads signaling pathway, Evid. Based Complement. Alternat. Med. 2017 (2017) 7187038.
[112] D.T. Wang, R.H. Huang, X. Cheng, Z.H. Zhang, Y.J. Yang, X. Lin, Tanshinone IIA attenuates renal fibrosis and inflammation via altering expression of TGF-β/Smad and NF-κB signaling pathway in 5/6 nephrectomized rats, Int. Immunopharm. 26 (2015) 4–12.
[113] T. Qin, S. Yin, J. Yang, Q. Zhang, Y. Liu, F. Huang, W. Cao, Sinomenine attenuates renal fibrosis through Nrf2-mediated inhibition of oxidative stress and TGFβ signaling, Toxicol. Appl. Pharmacol. 304 (2016) 1–8.
[114] L. Liu, Y. Wang, R. Yan, S. Li, M. Shi, Y. Xiao, B. Guo, Oxymatrine inhibits renal tubular EMT induced by high glucose via upregulation of SnoN and inhibition of TGF-β1/Smad signaling pathway, PLoS One 11 (2016) e0151986.
[115] J. Yang, M. Kan, G.Y. Wu, Bergenin ameliorates diabetic nephropathy in rats via suppressing renal inflammation and TGF-β 1-Smads pathway, Immunopharmacol. Immunotoxicol. 38 (2016) 145–152.
[116] J.J. Yoon, Y.J. Lee, D.G. Kang, H.S. Lee, Protective role of oryeongsan against renal inflammation and glomerulosclerosis in db/db mice, Am. J. Chin. Med. 42 (2014) 1431–1452.
[117] L. Wang, A.L. Cao, Y.F. Chi, Z.C. Ju, P.H. Yin, X.M. Zhang, W. Peng, You-gui Pill ameliorates renal tubulointerstitial fibrosis via inhibition of TGF-β/Smad signaling pathway, J. Ethnopharmacol. 169 (2015) 229–238.
[118] S. Liang, X. Meng, Z. Wang, J. Liu, H. Kuang, Q. Wang, Polysaccharide from *Ephedra sinica* Stapf inhibits inflammation expression by regulating Factor-β1/Smad2 signaling, Int. J. Biol. Macromol. 106 (2018) 947–954.
[119] H. Chu, Y. Shi, S. Jiang, Q. Zhong, Y. Zhao, Q. Liu, Y. Ma, X. Shi, W. Ding, X. Zhou, J. Cui, L. Jin, G. Guo, J. Wang, Treatment effects of the traditional Chinese medicine Shenks in bleomycin-induced lung fibrosis through regulation of TGF-β/Smad3 signaling and oxidative stress, Sci. Rep. 7 (2017) 2252.
[120] S.P. You, L. Ma, J. Zhao, S.L. Zhang, T. Liu, Phenylethanol glycosides from *Cistanche tubulosa* suppress hepatic stellate cell activation and block the conduction of signaling pathways in TGF-β1/smad as potential anti-Hepatic fibrosis agents, Molecules 21 (2016) 102.
[121] H.M. Liu, F. Dong, G.Q. Li, M. Niu, C.E. Zhang, Y.Z. Han, L.Z. He, P. Yin, B. Wang, X.X. Sang, R.S. Li, J.B. Wang, Z.F. Bai, X.H. Xiao, Liuweiwuling tablets attenuate BDL-induced hepatic fibrosis via modulation of TGF-β/Smad and NF-κB signaling pathways, J. Ethnopharmacol. 210 (2018) 232–241.
[122] H. Chen, H. Song, X. Liu, J. Tian, W. Tang, T. Cao, P. Zhao, C. Zhang, W. Guo, M. Xu, R. Lu, Buyanghuanwu Decoction alleviated pressure overload induced cardiac remodeling by suppressing TGF-β/Smads and MAPKs signaling activated fibrosis, Biomed. Pharmacother. 95 (2017) 461–468.