Transforming Growth Factor-β and Long Non-coding RNA in Renal Inflammation and Fibrosis

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Renal fibrosis is one of the most characterized pathological features in chronic kidney disease (CKD). Progressive fibrosis eventually leads to renal failure, leaving dialysis or allograft transplantation the only clinical option for CKD patients. Transforming growth factor-β (TGF-β) is the key mediator in renal fibrosis and is an essential regulator for renal inflammation. Therefore, the general blockade of the pro-fibrotic TGF-β may reduce fibrosis but may risk promoting renal inflammation and other side effects due to the diverse role of TGF-β in kidney diseases. Long non-coding RNAs (lncRNAs) are RNA transcripts with more than 200 nucleotides and have been regarded as promising therapeutic targets for many diseases. This review focuses on the importance of TGF-β and lncRNAs in renal inflammation, fibrogenesis, and the potential applications of TGF-β and lncRNAs as the therapeutic targets and biomarkers in renal fibrosis and CKD are highlighted.

Keywords: long non-coding RNA, renal fibrosis, inflammation, TGF-β, SMADs, molecular therapy

INTRODUCTION

Chronic kidney disease (CKD) has become a significant public health problem with the rising mortality and morbidity over the past three decades (Provenzano et al., 2019). Renal fibrosis is one of the most prominent pathogenic features and the best predictor for CKD progression (Majo et al., 2019). Triggered by the initial renal insults, the fibrotic process evolves to establish repairs. However, as severe or persistent injuries prolong, renal resident cells, together with infiltrating cells, may contribute to the initiation and progression of fibrosis with excessive deposition of extracellular matrix (ECM) in the glomerulus, tubulointerstitium, and vasculature (Glassock et al., 2017). Moreover, unresolved renal inflammation could also trigger the fibrotic process by releasing pro-fibrotic growth factors, cytokines, and chemokines (Chung and Lan, 2011; Meng et al., 2014). Injuries from mesangial cells, endothelial cells (ECs), podocytes, tubular epithelial cells (TECs), and inflammatory cells could also lead to renal glomerular and interstitial fibrosis (Figure 1). Progressive renal fibrosis and inflammation can then impair the function of nephrons and results in albuminuria and the reduction of eGFR. Renal fibrosis culminates in renal failure, well known as end-stage renal disease (ESRD) (Liu, 2011).
Transforming growth factor-β (TGF-β) is a primary pathophysiologic cytokine that instigates the process of fibrosis (Meng et al., 2016a). TGF-β can induce transcription of fibrotic products such as α-SMA and collagens by canonical and non-canonical signaling pathways. Fibrotic mediators include angiotensin II (Ang II), reactive oxygen species (ROS), as well as advanced glycation end products (AGEs) that may activate individual pathways to crosstalk with TGF-β/Smad signaling to regulate renal fibrosis and inflammation (Chung et al., 2010; Lan, 2011). However, current anti-fibrotic therapies by targeting TGF-β are ineffective with unexpected side effects, underscoring the complexities of the TGF-β signaling pathway (Yoshimura and Muto, 2011; Gu et al., 2020a).

With the new technologies of high-throughput assays, we can now update our understanding of the genomes. The transcriptomic studies have demonstrated that the vast majority of the genomes in mammals produce large numbers of non-protein-coding RNAs (ncRNAs) (Quinn and Chang, 2016). These ncRNAs are classified into long non-coding RNAs (lncRNAs), microRNAs (miRNAs), small interfering RNAs (siRNAs), small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), and PIWI-interacting RNAs (piRNAs) (Van der Hauwaert et al., 2019). Of these ncRNAs, lncRNAs are characterized as RNAs being transcribed over 200 nucleotides in length. They have been considered the major players in fibrotic diseases's pathogenesis due to their tissue and cell-type specificity and the regulations on DNAs, RNAs, and proteins (Jiang and Zhang, 2017). Of note, many TGF-β/Smad3-regulated lncRNAs have been reported as essential mediators in the process of renal fibrosis and inflammation (Tang et al., 2017, 2018a,b).

In this review, the underlying mechanistic signaling pathways by which TGF-β and lncRNAs drive renal fibrosis are to be discussed. The developments of biomarkers and therapeutic potential for renal inflammation and fibrosis by targeting TGF-β/Smad signaling and lncRNAs are also described.

DIVERSE ROLES OF TGF-β/SMAD SIGNALING PATHWAY IN RENAL INFLAMMATION AND FIBROSIS

Transforming growth factor-β is a pleiotropic cytokine that plays diverse roles in a wide range of biological and pathological processes. Indeed, TGF-β acts as either deleterious or protective
functions in kidney diseases (Lopez-Hernandez and Lopez-Novoa, 2012). TGF-β may induce renal fibrosis by canonical and non-canonical signaling pathways (Isaka, 2018). Besides, TGF-β promotes renal fibrosis by stimulating ECM accumulation and alternatively activating the pro-fibrotic immune cells, facilitating the transitions from various cell types into pro-fibrotic cells (Gu et al., 2020b). Therefore, understanding the diverse roles of TGF-β is of utmost importance in the development of anti-fibrotic therapies.

Transforming growth factor-β is a well-characterized member that belongs to the TGF-β superfamily. Among three isoforms of TGF-β, TGF-β1 is considered the pro-fibrotic molecule that drives the fibrotic process via canonical and non-canonical signaling pathways (Lodyga and Hinz, 2019). In particular, high expression of TGF-β1 is observed in most, not all progressive forms of human and rodent kidney diseases (Kopp et al., 1996; Fan et al., 1999; Lan, 2012b; Lan and Chung, 2012), demonstrating the pathogenic role for TGF-β1 in CKD. To induce transcriptions of target genes, likely as α-SMA and collagens, the latent TGF-β1 becomes active and binds to TGF-β receptors, promoting the transduction of a series of Smad proteins to regulate fibrogenesis (Derynck and Zhang, 2003). Regarding the downstream TGF-β/Smad signaling, although the functions of Smad2 and Smad4 have been well studied (Tsudoi et al., 2003; Ju et al., 2006; Meng et al., 2012; Morishita et al., 2014; Loeffler et al., 2018), their mechanistic roles are diverse and unclear due to the limited availability of animal models, which still warranted for further exploration.

It is widely acknowledged that Smad3 is pro-fibrotic, while Smad2 and Smad7 are anti-fibrotic. Smad3 is highly activated in a wide range of renal disease; evidence on animal models suggest that the inhibition or blockade of Smad3 may reduce the fibrotic response (Wang et al., 2006; Yang et al., 2009, 2010; Li et al., 2010; Zhou et al., 2010; Liu et al., 2012; Zhang et al., 2018). By contrast, the function of Smad2 and Smad7 is protective, which negatively regulates the TGF-β/Smad3 signaling in renal fibrosis and inflammation (Lan, 2008, 2012a; Chen et al., 2011). Many studies support this finding, showing that overexpression of Smad7 improves renal fibrogenesis in obstructive, diabetic, hypertensive, toxin-induced nephropathy and autoimmune crescentic glomerulonephritis (Li et al., 2002; Lan et al., 2003; Hou et al., 2005; Ng et al., 2005; Ka et al., 2007; Chung et al., 2009; Liu et al., 2013, 2014; Dai et al., 2015) by inhibiting the TGF-β/Smad3 and NF-κB signaling pathways. However, the contradictory findings have also reported that the overexpression of Smad7 could promote TGF-β-driven apoptosis in podocytes (Schiffer et al., 2001, 2002). Collectively, although restoring the imbalance between Smad3 and Smad7 may serve as an ideal therapy to halt the fibrotic process (Nie et al., 2014; Zhao et al., 2014, 2016; Meng et al., 2015; Du et al., 2018b), Smad3 and Smad7 also serve as the vital downstream molecules in other signaling pathways. Therefore, new specific targets should be sought.

Transforming growth factor-β may also be produced by damaged renal intrinsic cells or immune cells in acute and CKDs, thus promoting the transition of tubular cells into myofibroblasts (Mack and Yanagita, 2015). Myofibroblasts produce fibronectin and collagens and contribute to ECM accumulation (Yuan et al., 2019). Based on current studies, the sources of myofibroblast origins include pericytes (Wu et al., 2013), renal resident fibroblasts, tubular epithelial cell-myofibroblast transition (EMT) (Iwano et al., 2002), endothelial cell-myofibroblast transition (EndoMT) (Zeisberg et al., 2008) and bone marrow-derived macrophage-myofibroblast transition (MMT) (Fan et al., 1999; Meng et al., 2016b; Wang et al., 2017). TGF-β/Smad signaling pathway tightly regulates these transitions.

To halt the fibrotic process, strategies to inhibit the function of TGF-β include the utilization of neutralizing antibodies (Border et al., 1990), small molecule inhibitors against TGF-β receptors (Bonafox and Lee, 2009), latent form of TGF-β (Huang et al., 2008a,b) and antisense oligonucleotides to TGF-β1 (March et al., 2018). These findings have conferred a vital pathological role of TGF-β in renal inflammation and fibrosis, implying the urgent need for anti-TGF-β therapy.

**THERAPEUTIC EFFECT OF ANTI-TGF-β TREATMENT ON KIDNEY DISEASES**

Anti-TGF-β therapy is an issue of considerable debate. On the one hand, TGF-β is the crucial mediator that regulates fibrosis in all organs, especially in kidneys (Györfi et al., 2018). On the other hand, TGF-β regulates a wide range of biological and pathological processes and acts as essential roles in the immune cells, such as macrophages, conventional and unconventional T cells (Meng, 2019; Gu et al., 2020a). Over the past decades, a number of therapeutic drugs and clinical trials for the treatment of CKD targeting TGF-β have further revealed the underlying mechanisms and renewed our understanding of TGF-β signaling (Ruiz-Ortega et al., 2020).

Targeting on the TGF-β family, LY2382770 and fresolimumab have proven no efficacy on improvements in neither proteinuria, eGFR, nor serum creatinine in focal and segmental glomerulosclerosis (FSGS) and diabetic nephropathy (DN) (Trachtman et al., 2011; Vincent et al., 2017; Voelker et al., 2017). Besides, various side effects induced by blocking TGF-β, including herpes zoster, skin lesions, pustular rash, bleeding events, and cancers, have demonstrated the awkward situation of the anti-TGF-β therapies. Hopefully, with the rapid development of pharmacology, a promising synthetic anti-TGF-β agent, pirfenidone, is proven to improve the eGFR decline in patients with DN and FSGS (Cho et al., 2007; Sharma et al., 2011). Further studies and clinical trials on pirfenidone’s renal protective effects are still ongoing (NCT02689778, NCT02408744, and NCT00001959).

Nevertheless, the by-effects such as gastrointestinal disorders and photosensitive dermatitis of pirfenidone are inevitable, raising safety concerns to the clinical application of anti-TGF-β therapies. Current anti-TGF-β therapies have limited effectiveness, underscoring the urgent need to develop specific therapeutic targets to halt the progression of renal fibrosis.
**THE EMERGING ROLE OF LONG NON-CODING RNAs IN RENAL INFLAMMATION AND FIBROSIS**

The genomic and transcriptional landscape is far more complicated than we previously appreciated. With the development of large-scale transcriptome analyses, we have now acknowledged that the vast majority of genomic sequence is transcribed into a group of IncRNAs (Hangauer et al., 2013). However, these IncRNAs were initially ignored as “transcriptional noise” or “evolutionary debris,” dating from the 1970s (Ohno, 1972). In the 1990s, the functions of some classically defined IncRNAs are discovered, such as X inactive specific transcript (XIST) in X chromosome inactive specific, raising the possibility that IncRNAs may play an essential role in cellular biology and disease (Brockdorff et al., 1991; Brown et al., 1991). Of note, with the emergence of IncRNAs, the number of identified lncRNAs is rapidly rising to date.

In the 1970s, Ohno (1972) hypothesized that the vast majority of genomic sequence is transcribed into “RNA noise” or “evolutionary debris,” making the function of IncRNAs largely unknown. However, with the advancement of large-scale transcriptome analyses, it is now acknowledged that the vast majority of genomic sequence is transcribed into IncRNAs (Hangauer et al., 2013). In the 1990s, the functions of some classically defined IncRNAs (e.g., X inactive specific transcript (XIST)) are discovered in the context of X chromosome inactive specific, raising the possibility that IncRNAs may play an essential role in cellular biology and disease (Brockdorff et al., 1991; Brown et al., 1991).

**Pathological hallmarks** include TGF-β and cytokines produced by inflammatory immune cells may activate innate and acquired immune response (Gu et al., 2020b). These may be associated with the functions of IncRNAs in renal inflammation. For instance, IncRNAs may act as mediators in lupus nephritis pathogenesis to regulate inflammation and apoptosis of renal cells (Xue et al., 2017; Liao et al., 2019; Chen et al., 2020). Nevertheless, IncRNAs take part in the fibrotic or inflammatory transcriptional regulation by direct interactions with RNA polymerase II (Pol II), transcription factors (TFs), and other regulators. Furthermore, some IncRNAs may act as competing endogenous RNAs (ceRNAs), which play the competitive role as the sponges to bind with miRNAs and reduce the concentration of fibrotic or inflammatory miRNAs, therefore competing with these miRNAs in binding to their target mRNA transcripts.

As previously mentioned, the group of IncRNAs identified in kidneys is highly specific to cell type or disease state. Studies carried out over these years have identified a group of anti- or pro-fibrotic and inflammatory IncRNAs in diabetic, acute and chronic renal diseases (Tang et al., 2017, 2018a; Ren et al., 2019; Gu et al., 2020c) (Table 1).

For example, hyperglycemia is one of the most driving forces in renal fibrosis. Zhang et al. has revealed the anti-fibrotic effect of IncRNA growth arrest-specific transcript (GASS) in the progression of DN. IncRNA GASS may downregulate the expression of pro-inflammatory MMP9 by recruiting EZH2 to the MMP9 promoter region, therefore inhibiting renal interstitial fibrosis and inflammatory (Zhang et al., 2020a). IncRNA CRNDE also interacts with miR-181a-5p to to protect sepsis-induced AKI from apoptosis (Wang et al., 2020a). Moreover, overexpression of IncRNA CCAT1 may down-regulate miR-155, thus inhibiting inflammation and promoting proliferation (Lu et al., 2020). LncRNA zinc finger E-box binding homeobox-1-antisense RNA 1 (ZEB1-AS1) provides a binding site in its promoter region for p53. It may promote H3K4me3 histone modification on ZEB1 promoter to exhibit anti-fibrotic effect (Wang et al., 2018a). In the context of fibrosis, the function of IncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) has been well-studied in cardiac and in hepatic fibrosis (Jiang et al., 2019; Riaz and Li, 2019; Che et al., 2020). MALAT1 has caught much attention in renal diseases for its anti-inflammatory effect in AKI. The expression of LncRNA 1700020I14Rik tends to decrease under high glucose conditions, but the overexpression of LncRNA 1700020I14Rik exerts an anti-fibrotic effects by inhibiting cell proliferation and regulating the miR-20a-5p/Sirt1/HIF-1α pathway (Li et al., 2018). Moreover, LncRNA CYP4B1-PS1-001 significantly reduces in the early stage of DN; the proliferation and fibrosis of mesangial cells are reversed as the overexpression of CYP4B1-PS1-001 regulates the ubiquitination and degradation of Nucleolin (Wang et al., 2016a, 2018c). ENSMUST00000147869 is significantly downregulated in the DN model. Overexpression of ENSMUST00000147869 may inhibit fibrosis and proliferation of mesangial cells by the possible regulation of the Cyp4a12a gene (Wang et al., 2016b). To interact with miRNAs and proteins in podocytes, pericytes, or TECs, IncRNAs such as taurine upregulated gene 1 (TUG1) (Zhao et al., 2019; Cao et al., 2020a,b), Rian (Bijkerk et al., 2019), 3110045C21Rik (Arvaniti et al., 2016) also function as competing endogenous RNAs (ceRNAs), which play the competitive role as the sponges to bind with miRNAs and reduce the concentration of fibrotic or inflammatory miRNAs.

Studies on pro-fibrotic IncRNAs are shown in Table 2. LncRNA myocardial infarction-associated transcript (Miat) has been identified to function as miRNA sponges in TECs and pericytes, thus regulating their transitions into myofibroblast (Bijkerk et al., 2019; Wang et al., 2020b). In diabetes-induced renal injury, IncRNA nuclear enriched abundant transcript 1 (NEAT1) is found to be increased in the serum of DN patients. A further mechanistic study has revealed that IncRNA NEAT1 may progress the development of DN by sponging miR-23c.
Moreover, studies from Yang et al. and Huang et al. have drawn similar conclusions. At the same time, they further investigated that NEAT1 may also promote renal inflammation in lupus nephritis by upregulating the expression of TRAF6 and activating the NF-κB signaling in lupus nephritis (Zhang et al., 2020b). It is reported that NEAT1 may also drive the progression of fibrosis under diabetic conditions. Furthermore, lncRNA Gm4419 is increased in DN and promotes renal fibrosis and inflammation by activating inflammatory cytokines, together with NLRP3 inflammasome, may also drive the progression of fibrosis under diabetic conditions. Additionally, microarray data have shown a pro-fibrotic role of lncRNA LINC00963 by targeting on FoxO3 gene to regulate the FoxO signaling pathway (Yi et al., 2017). However, no significant differences in inflammation and fibrosis were shown on MALAT1 knockout and wild-type mice in hypoxia-induced AKI (Kölling et al., 2018).

Also, microarray data have shown a pro-fibrotic role of lncRNA LINC00963 by targeting on FoxO3 gene to regulate the FoxO signaling pathway (Chen et al., 2018). Pro-inflammatory cytokines, together with NLRP3 inflammasome, may also drive the progression of fibrosis under diabetic conditions. Furthermore, IncRNA Gm4419 is increased in DN and promotes renal fibrosis and inflammation by activating the NF-κB/NLRP3 inflammasome signaling pathway in MCs (Yi et al., 2017). However, the functional roles of IncRNA ENSRNOG00000037522 and CHCHD4P4 remain to be further investigated (Zhang et al., 2017a; Ling et al., 2018).

| IncRNA   | Model                       | Mechanism/target                                      | Pathological output(s)         | Year   | References          |
|----------|-----------------------------|-------------------------------------------------------|--------------------------------|--------|---------------------|
| GAS5     | STZ-induced DN and rat      | Recruits EZH2 to the promoter region of MMP9           | Anti-fibrotic; anti-inflammatory | 2020   | Zhang et al., 2020a |
| CRNDE    | Sepsis-induced AKI, rat, and TECs | Regulation of miR-181a-5p/PPARα pathway               | Anti-inflammatory               | 2020   | Wang et al., 2020a  |
| CCAT1    | LPS-induced AKI mice and TECs | Overexpression of CCAT1 and leads to upregulation of SIRT1 and TECs damage | Anti-inflammatory               | 2020   | Lu et al., 2020     |
| TUG1     | SLE patient serum and SLE mouse | /                                                     | Anti-fibrotic; anti-inflammatory | 2020   | Cao et al., 2020a,b |
| Rian/RIAN| LPS-induced podocyte injury | Targets miR-197/MAPK1                                  | Anti-inflammatory               | 2019   | Zhao et al., 2019   |
| Malat1/MALAT1 | AKI; mice, and TECs       | Regulates HIF-1α expression through NF-κB signaling   | Anti-inflammatory               | 2018   | Tian et al., 2018   |
| ZEB1-AS1 | DN mouse and DN patient    | Binds to H3K4 methyltransferase and MLL1 to promote ZEB1 expression | Anti-fibrotic                   | 2018   | Wang et al., 2018a  |
| 1700020H14Rik | DN mouse and MCs          | Interacts with miR-34a-5p, Sirt1/HIF-1α                | Anti-fibrotic                   | 2018   | Li et al., 2018     |
| CYP4B1-PS1-001 | DN mouse and MCs         | Regulates Nucleolin to inhibit proliferation and fibrosis of MCs | Anti-fibrotic                   | 2016   | Wang et al., 2016a, 2018c |
| 3110045C21Rik | UUO mouse and TECs        | Contains binding sites for Pol II and H3K4m3           | Anti-fibrotic                   | 2016   | Arvanti et al., 2016 |
| ENSMUST00000147869 | DN mouse and MCs     | Possibly targets on Cyp4a12a gene                      | Anti-fibrotic                   | 2016   | Wang et al., 2016b  |

In summary, IncRNA MALAT1 upregulation in TECs and podocytes under high glucose-induced conditions. Induced by TGF-β1, MALAT1 facilitates EMT and promotes fibrosis by acting as a sponge for miR-145 or as a feedback regulator of the Wnt/β-catenin signaling pathway (Hu et al., 2017; Liu et al., 2019a; Zhang et al., 2019a). However, the pathogenic role of IncRNA MALAT1 in hypoxia-induced AKI remains unclear. Kölling et al. have identified an increased level of IncRNA MALAT1 in renal biopsies and plasma of AKI patients; in vitro study has also shown a decreased number and proliferation in MALAT1-inhibited ECs. The mechanistic study has discovered that it is transcriptionally activated by hypoxia-inducible factor 1α (HIF-1α). However, no significant differences in inflammation and fibrosis were shown on MALAT1 knockout and wild-type mice in hypoxia-induced AKI (Kölling et al., 2018).

**TRANSFORMING GROWTH FACTOR-β/SMAD3-DEPENDENT LNCRNA IN RENAL INFLAMMATION AND FIBROSIS**

Fibrotic responses triggered by TGF-β/Smad3 signaling are of importance in renal fibrogenesis. However, generally blocking the
upstream TGF-β signaling may risk promoting inflammation and other side effects. We are beginning to learn that the involvement of TGF-β in many other biological processes has been the main obstacle for anti-TGF-β therapy. Nevertheless, the majority of studies continue to seek therapeutic targets for anti-fibrotic treatments. miRNA targeting downstream TGF-β signaling has been one of the optimal options.

However, the off-target effects and cytotoxicity of miRNA therapies have caught the attention of their specificity and safety. Encouragingly, it has been reported that a group of characterized lncRNAs is involved in TGF-β/Smad3-mediated renal fibrosis and inflammation (Zhou et al., 2014, 2015b) (Table 3). These emerging studies should provide possibilities for lncRNA treatment in the future. Ptprd-IR is a novel lncRNA that promotes inflammatory response on TECs in the UUO model. It contains a binding site for Smad3 in its promoter region and is downregulated by deleting Smad3. In contrast, the overexpression of Ptprd-IR enhances inflammatory response by upregulating TGF-β1-, interleukin-1β (IL-1β)-induced NF-κB-driven production of pro-inflammatory cytokines but shows no effect on the TGF-β1-induced renal fibrosis (Pu et al., 2020).

Other novel lncRNA, lncRNA Erbb4-IR, of which expression is induced by TGF-β1 via Smad3-dependent mechanism, is significantly increased in the fibrotic UUO model (Feng et al., 2018). Erbb4-IR binds to the inhibitory Smad7 and blocks TGF-β/Smad3-induced renal fibrosis, while overexpression of Erbb4-IR may promote fibrosis by downregulating the expression of Smad7. Of note, Erbb4-IR may also be induced by advanced

| lncRNA       | Model                               | Mechanism/function                                      | Pathological output(s) | Year  | References                  |
|--------------|-------------------------------------|--------------------------------------------------------|------------------------|-------|-----------------------------|
| Miat/MIAT    | UUO mouse and TECs                  | Sponge for miR-145                                     | Pro-fibrotic           | 2020  | Wang et al., 2020b          |
| Neat1/NEAT1  | DN mouse and TECs                   | Regulates the Klotho/ERK1/2 signaling                  | Pro-fibrotic           | 2020  | Yang et al., 2020          |
|              | Plasma from DN patient, DN mouse,   | Sponge for miR-23c                                     | Pro-fibrotic           | 2020  | Li et al., 2020             |
|              | and MCs                             | Possible regulation of Akt/mTOR                        | Pro-fibrotic           | 2019  | Huang et al., 2019          |
|              | DN rat and MCs                      | Targets miR-146b to promote TRAF6 expression           | Pro-fibrotic           | 2019  | Zhang et al., 2020b         |
| LOC105375913 | FSOS patient and TECs               | Regulated by C3ap38/XBP-1s signaling and binds to miR-27b| Pro-fibrotic           | 2019  | Han et al., 2019            |
| LINC00667    | CKD patient, CKD rat, and TECs      | Promotes fibrosis via miR-19b-3p/LINC00667/CTGF signaling| Pro-fibrotic           | 2019  | Chen et al., 2019           |
| TapSAKI      | Sepsis-induced AKI; rats; and TECs  | Promotes apoptosis and inflammation of TECs via TaqSAKI/miR-22/TLR4/NF-κB signaling pathway | Pro-inflammatory       | 2019  | Shen et al., 2019           |
| Rpph1        | db/db mice and MCs                  | Promotes inflammation and MCs proliferation through Gal-3/Mek/Erk signaling | Pro-inflammatory       | 2019  | Zhang et al., 2019b         |
| Bnc1         | DN patient, STZ-induced DN, and TECs| Interaction with NRF2/HO-1 and NF-κB signaling          | Pro-fibrotic; Pro-inflammatory | 2019  | Feng et al., 2019           |
| Malat1/MALAT1| DN and TECs                         | Regulation of Wnt/β-catenin signaling                  | Pro-fibrotic; Pro-inflammatory | 2019  | Zhang et al., 2019a         |
|              | DN mouse and TECs                   | Sponge for miR-145                                    | Pro-fibrotic; Pro-inflammatory | 2019  | Liu et al., 2019a           |
|              | Plasma, renal biopsies from AKI     | Regulated by HIF-1α                                     | No significant effect  | 2018  | Kölling et al., 2018        |
|              | patients, IRI mouse, TECs, and ECs  | Binds to SRSF1, interacts with β-catenin               | Pro-fibrotic           | 2017  | Hu et al., 2017             |
|              | DN mouse and podocytes              | Upregulated IL-6, TNF-α by activating SAA3             | Pro-inflammatory       | 2015  | Puthanveetil et al., 2015   |
|              | STZ-induced mice and ECs            | /                                                      | Pro-fibrotic           | 2015  | Liu et al., 2018            |
|              | DNPND400000037522                   | Negatively regulates miR-743b-5p/5p                    | Pro-fibrotic           | 2018  | Gao et al., 2018            |
|              | NR_033515                           | Activates the FoxO signaling                           | Pro-fibrotic           | 2018  | Chen et al., 2018           |
|              | LINC00963                           | Kidney stone, mouse and TECs                          | /                      | 2017  | Zhang et al., 2017a         |
|              | Gm4419                              | DN mouse and MCs                                      | Upregulated by ROS     | 2017  | Gao et al., 2017            |
|              | PVT1                                | AKI; and LPS-induced TECs                              | Binds to TNF-α and inhibits JNK/ NF-κB signaling pathway | Pro-inflammatory | 2017  | Huang et al., 2017          |
|              | RP23-45G16.5                        | UUO mouse and TECs                                    | Shows positive correlation with cdkn1b gene               | Pro-fibrotic | 2016  | Anvari et al., 2016        |

TABLE 2 | Pro-fibrotic or pro-inflammatory lncRNAs in renal diseases.
glycosylation end products (AGEs) in DN. It promotes the expression of collagens by binding to miR-29b and hence transcriptionally suppresses miR-29b. Silencing renal ErbB4-IR leads to the upregulation of protective miR-29b and prevents fibrosis (Sun et al., 2018; Xu et al., 2020). Besides, IncRNA AT-rich interactive domain 2-IR (Arid2-IR) also contains a Smad3 binding site in the promoter region. Further in vivo study has shown that deletion of Smad3 may abolish upregulation of Arid2-IR in the diseased kidney. Arid2-IR shares a similar mechanism with Ptprd-IR that overexpression of Arid2-IR may promote TGF-β1-, IL-1β-induced NF-κB-driven inflammation without affecting TGF-β/Smad3-mediated renal fibrosis (Zhou et al., 2015a). Nevertheless, the study from Yang et al. (2019) has demonstrated the upregulation and pro-fibrotic effect of Arid2-IR on MCs in DN, that Arid2-IR may be positively regulated by the early growth response protein-1 (Egr1) and promote ECM production.

A novel Smad3-dependent IncRNA, LRNA9884, is induced by AGEs and tightly regulated by Smad3 in the development and progression of DN. Mechanistically, LRNA9884 directly binds to MCP-1 and enhances the promoter activity of MCP-1 at the transcriptional level, thus aggravating the renal injury driven by progressive inflammation (Zhang et al., 2019c). The kidney-enriched TGF-β/Smad3-interacting IncRNA, term as Inc-TSI, is another novel IncRNA that serves as a potential target for renal fibrosis (Wang et al., 2018b). Inc-TSI inhibits renal fibrosis by binding to the MH2 domain of Smad3, therefore blocking the interaction of Smad3 and TβRII and inhibiting the phosphorylation of Smad3. Meanwhile, the overexpression of Inc-TSI prevents the nuclear translocation of Smad2/3/4, resulting in the decreased expression of fibrotic proteins. The anti-fibrotic role of Inc-TSI has further confirmed that the fibrosis index of IgAN patients is negatively correlated with the expression of Inc-TSI.

Collectively, the TGF-β/Smad3-mediated IncRNAs may act as anti-fibrotic or pro-fibrotic mediators in the fibrotic process by binding to Smad3, Smad7, or inflammatory molecules to inhibit or enhance renal fibrosis and inflammation. It

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**TABLE 3 | TGF-β/Smad3-dependent IncRNAs in renal fibrosis and inflammation.**

| ncRNA          | Model                                           | Mechanism/function                                      | Pathological output(s)     | Year     | References                  |
|----------------|-------------------------------------------------|---------------------------------------------------------|-----------------------------|----------|-----------------------------|
| Ptprd-IR (np_4334) | UUO mouse and TECs | Contains a binding site for Smad3 and promotes NF-κB-driven inflammation | Pro-inflammatory          | 2020     | Pu et al., 2020             |
| Erbb4-IR (np_5318) | DN mouse, TECs, and MCs | Binds to miR-29b to downregulate miR-29b expression | Pro-fibrotic               | 2020     | Sun et al., 2018; Xu et al., 2020 |
| Arid2-IR (np_28496) | DN mouse and MCs | Contains a binding site for Smad3 and promotes NF-κB-driven inflammation | Pro-inflammatory          | 2015     | Zhou et al., 2015a          |
| LRNA9884 | DN mouse, TECs, and MCs | Directly triggers the MCP-1 production | Pro-inflammatory          | 2019     | Zhang et al., 2019c         |
| NONHSAG053901 | DN mouse and MCs | Directly binds to Egr-1 | Pro-fibrotic; pro-inflammatory | 2019     | Peng et al., 2019           |
| HOTAIR | UUO rat and TECs | Regulation of miR-124 /Notch1 | Pro-fibrotic               | 2019     | Zhou et al., 2019           |
| lncRNA-ATB | UUO rat and TECs | Regulated by Lvnin to promote EMT | Pro-fibrotic               | 2019     | Zhou and Jiang, 2019        |
| MEG3 | TECs | Regulated by miR-185/DNMT1 axis to inhibit fibrosis | Anti-fibrotic              | 2019     | Xue et al., 2019            |
| ENST00000453774.1 | Human renal fibrotic tissue, UUO mouse, and TECs | Activates autophagy by promoting ROS defense activates Hif2/HO-1 signaling | Anti-fibrotic              | 2019     | Xiao et al., 2019           |
| Inc-TSI | IgAN patient and UUO mouse | Binds with Smad3 to block the interaction between Smad3 and TβRI | Anti-fibrotic              | 2018     | Wang et al., 2018b          |
| TCONS_0008786 | UUO mouse and TECs | Possible regulation of miR-132 | Pro-fibrotic               | 2018     | Zhou et al., 2018           |
| TCONS_01496394 | UUO rat and TECs | / | Pro-fibrotic               | 2017     | Sun et al., 2017            |
| H19 | UUO mouse, DN mouse, and TECs | Stimulated by TGF-β2 and serves as a sponge for miR-17 | Pro-fibrotic               | 2016     | Xie et al., 2016            |

CKD, chronic kidney disease; FSGS, focal and segmental glomerulosclerosis; STZ, streptozocin; DN, diabetic nephropathy; SLE, systemic erythematosus lupus; IgAN, IgA nephropathy; anti-GBM, anti-glomerular basement membrane; LPS, lipopolysaccharides; UUO, unilateral ureteral obstruction; AKI, acute kidney injury; IRI, ischemia-reperfusion injury; MCs, mesangial cells; TECs, tubular epithelial cells; ECs, endothelial cells; SAA3, serum amyloid antigen 3.
has been demonstrated by a large number of studies that lncRNAs act like an endogenous RNA to compete for miRNA to regulate the target transcripts at the transcriptional or post-transcriptional level during renal fibrosis. In the early stage of DN, the expression of lncRNA NONHSAG053901 is highly increased in DN mice and MCs. The functional study has revealed that the overexpression of NONHSAG053901 promotes fibrosis, inflammation, and proliferation in MCs. Mechanistically, NONHSAG053901 directly binds to Egr-1, which later interacts with TGF-β to upregulate the release of pro-inflammatory cytokines to promote Egr-1/TGF-β mediated renal inflammation (Peng et al., 2019). In addition, the pro-fibrotic lncRNA HOTAIR is significantly upregulated in TGF-β-induced inflammation (Gu et al., 2019). The functional study has revealed that the overexpression of NONHSAG053901 highly increased in DN mice and MCs. The functional study has revealed that the overexpression of NONHSAG053901 promotes fibrosis, inflammation, and proliferation in MCs. Mechanistically, NONHSAG053901 directly binds to Egr-1, which later interacts with TGF-β to upregulate the release of pro-inflammatory cytokines to promote Egr-1/TGF-β mediated renal inflammation (Peng et al., 2019). In addition, the pro-fibrotic lncRNA HOTAIR is significantly upregulated in TGF-β1-induced TECs and UUO rat kidney. Depletion on HOTAIR upregulates miR-124 to block the Notch1 signal pathway, therefore improving the EMT and reducing the accumulation of fibrotic proteins such as fibronectin and α-SMA (Zhou et al., 2019).

Inc-ATB has also been proven to be the critical regulator stimulated by TGF-β that mediates the EMT process. The expression of IncRNA-ATB is significantly increased in TECs and the UUO kidney under TGF-β and Livin regulation (Zhou and Jiang, 2019). Another lncRNA regulated by TGF-β is maternally expressed gene 3 (MEG3), inhibited in TGF-β-stimulated TECs. DNA methyltransferases 1 (DNMT1), regulated by miR-185, can positively modulate the methylation state of CpG islands in the promoter region of MEG3. Overexpression of lncRNA MEG3 reverses TGF-β-induced fibrosis in TECs. Thus, lncRNA MEG3 exerts an anti-fibrotic effect in TGF-β-promoted EMT and is regulated by the miR-185/DNMT1 signaling pathway (Xue et al., 2019). However, one study had investigated the pro-inflammatory effect of MEG3 in the acute renal allograft model (Pang et al., 2019). The anti-fibrotic lncRNA, ENST00000453774.1, is also downregulated in TGF-β-induced TECs and UUO model, especially in the fibrotic renal biopsies from patients. ENST00000453774.1 may regulate the Nrf2-keap1/Nrf2 nuclear translocation/HO-1 and NQO-1 signaling to activate the pro-survival autophagy of TECs, therefore promoting ROS defense and reducing the production of ECM markers such as fibronectin and collagen I (Xiao et al., 2019).

Nevertheless, the mechanism of some pro-fibrotic lncRNAs is still obscure. Based on the transcriptome sequencing study, a group of lncRNAs that contain Smad3 binding motifs in the promoter region has been identified. Among these lncRNAs, TCONS_00088786 and TCONS_01496394 are confirmed to be regulated by TGF-β in a time and dose-dependent manner. Knockdown of TCONS_00088786 may inhibit the mRNA expression profile of the gene Acta1, Col1a1, and Col3a1, while knockdown of TCONS_01496394 decreases the mRNA expression of Ctgf and Fln1, suggesting their potential in promoting renal fibrosis (Sun et al., 2017). Although a functional study has shown a positive regulation of TCONS_00088786 on miR-132, the underlying mechanism is unclear (Zhou et al., 2018). Interestingly, the expression of lncRNA H19 is also increased in TECs and the UUO model. lncRNA H19 is activated in embryonic cells, but its expression is significantly decreased after birth. Under the renal fibrotic condition, H19 is upregulated by TGF-β2 to promote the production of ECM-related proteins. Knockdown of H19 restores the renal functions and inhibits TGF-β2-induced fibrosis. It is demonstrated that H19 serves as a sponge for miR-17 and negatively regulates miR-17 in the process of fibrogenesis (Xie et al., 2016). However, further evidence on how H19 and miR-17 contribute to the network of renal fibrosis remains unclear.

**FUTURE PERSPECTIVES: LncRNA AS A NOVEL THERAPEUTIC TARGET FOR KIDNEY DISEASE**

The activation of TGF-β/Smad signaling is one of the most characterized features in fibrosis. Although TGF-β is the crucial driver of fibrotic response, it also acts as an anti-inflammatory cytokine and essential mediator that regulates a wide range of biological processes in different cell types and disease conditions. Numerous studies reveal that lncRNAs participate in the emergence and progression of kidney diseases. An outline is becoming manifest in the contribution of TGF-β/Smad-mediated lncRNAs in renal fibrogenesis.

We are now getting better closer to understand how these lncRNAs regulate fibrosis. They can bind to the Smads proteins to exert either anti- or pro-fibrotic effects. They can also serve as miRNA sponges and interact with other signaling pathways to regulate ECM accumulation, EMT, MMT, or other fibrotic processes.

Based on the cell type-, tissue- and disease stage-dependent specialties, lncRNA may also present as biomarkers for clinical diagnosis in renal diseases (Brandenburger et al., 2018; Cheng et al., 2019; Li et al., 2019; Liu et al., 2019b; Loganathan et al., 2020). Interestingly, lncRNAs are relevant biomarkers for disease due to their existence with proteins or in vesicles in the extracellular space under pathological conditions (Teng and Ghoshal, 2015; Ellinger et al., 2016; Zhang et al., 2017c; E, S., Costa et al., 2018; Sarfi et al., 2019). Studies have demonstrated that circulating lncRNAs in body fluid, lncRNA GAS8-AS1, H19, metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), and HOTAIR may be used as promising biomarkers to predict the early progression of cancers (Zhang et al., 2016, 2017b; Du et al., 2018a). Notably, the lncRNA expression profiles in urine also contribute to the early detection of acute T cell-mediated rejection of renal allografts (Lorenzen et al., 2015b), highlighting the importance of lncRNAs in T cell-mediated immune response during renal injuries (Hu et al., 2013).

The modulation of lncRNAs on renal fibrosis is a promising therapeutic target for fibrosis. However, it remains largely unexplored. The low expression amounts, the less conservation between species, the functional complexity, and the difficulty in modifying structures and locations of lncRNA in nuclear or cytoplasmic compartments have halted the development of lncRNA therapies.

Nevertheless, new technologies such as CRISPR/Cas9 editing (Wang et al., 2019; Horlbbeck et al., 2020), Gapmer antisense
oligonucleotide-mediated lncRNA silencing (Castanotto et al., 2015; Kuespert et al., 2020), plasmid/vector-delivery short hairpin RNAs (shRNAs) (Zhu et al., 2019; Yao et al., 2020) and ultrasound-mediated gene transfer method (Zhou et al., 2015a; Feng et al., 2018; Sun et al., 2018; Zhang et al., 2019c) may represent the novel strategies to modulate the expression and function of lncRNA in kidney diseases in the future.

**AUTHOR CONTRIBUTIONS**

Y-YG, J-YD, and X-RH wrote the manuscript, X-SL and H-YL revised and edited the manuscript. All authors contributed to the discussion of this manuscript.

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Conflicts of Interest: The authors declare that the research was conducted in the
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