TGF-β1 Signaling in Kidney Diseases

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Transforming growth factor-β (TGF-β) is a crucial pathogenic mediator of inflammatory diseases. In tissue fibrosis, TGF-β regulates the pathogenic activity of infiltrated immunocytes and promotes extracellular matrix production via de novo myofibroblast generation and kidney cell activation. However, TGF-β is highly pleiotropic in tissue fibrosis, and thus, direct targeting of TGF-β may also block its protective anti-inflammatory effects, resulting in undesirable outcomes. Increasing evidence suggests the involvement of long non-coding RNAs (lncRNAs) in TGF-β-driven tissue fibrosis with a high cell-type and disease specificity, serving as an ideal target for therapeutic development.

Keywords: long non-coding RNA ; fibrosis ; transforming growth factor-β

1. Introduction

Long non-coding RNAs (lncRNAs) are transcripts with lengths of over 200 nucleotides that together with short microRNAs (miRNAs), small interfering RNAs (siRNAs), small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs), and PIWI-interacting RNAs (piRNAs) constitute a spectrum of non-coding RNA molecules (ncRNAs) characterized by their gene-regulating functions [1][2][3]. Of these, lncRNAs and miRNAs are two major classes of ncRNAs that participate in the pathogenesis of cancer and fibrotic diseases, as dysregulation of lncRNAs and miRNAs interferes with the control of crucial biological processes, including cell proliferation, apoptosis, and extracellular matrix homeostasis [4][5]. Since their discovery with high-throughput RNA sequencing, various biological functions of lncRNAs have been revealed, suggesting that these RNAs may be a missing piece of a complex gene-regulatory mechanism that supports higher life forms and not junk transcripts left over from evolution [6][7]. Emerging evidence indicates that lncRNAs are important for fine-tuning transcription, contributing to the regulatory network of spatial and temporal gene expression with high specificity. Thus, lncRNAs may represent an optimal target for diagnosing and treating diseases.

Transforming growth factor-β (TGF-β) is a crucial cytokine that drives the pathogenesis and development of chronic inflammatory diseases, particularly chronic kidney disease (CKD) and cancer [8][9][10][11]. TGF-β signaling is highly activated in experimental models and in patient biopsies of CKD associated with enhanced production and deposition of extracellular matrix (ECM) components like collagens and fibronectins, contributing to the disruption of tissue structure and eventually leading to end-stage renal disease (ESRD) with complete loss of function [12][13]. However, fibrotic response in a resolvable manner is an essential mechanism for repairing initial tissue injury. Therapy directly targeting TGF-β may lead to side effects in vital organs [14]. This scenario also applies to cancer in that pleiotropic cytokine TGF-β initially suppresses proliferation and induces apoptosis of cancer cells but promotes immunosuppression, angiogenesis, and cancer cell growth via stromal cells in established tumors [15][16]. Therefore, identifying pathogenic downstream mediators of TGF-β is essential for developing a specific strategy that suppresses the pathogenic activity while preserving the therapeutic and physiological activities of TGF-β. TGF-β induced lncRNAs with high spatial and temporal specificity in the pathogenesis of inflammatory disease, serving as an ideal target for developing targeted TGF-β signaling therapy [1][17][18].

2. TGF-β1 Signaling in Kidney Diseases

TGF-β1 is primarily involved in a dynamic pathophysiological process that leads to renal fibrosis. It is significantly upregulated in the injured kidney as a primary step in tissue scarring [8][19][20] and the progressive forms of kidney disease [21]. The latest studies have revealed the diverse roles of TGF-β1, for instance as a major inducer of macrophage polarization [22], myofibroblast differentiation, and accumulation in the fibrotic kidney, which is primarily reduced by conditional deletion of TGFβ receptor 2 (Tgfbr2) [23][24][25].

TGF-β1 acts on both residential kidney cells (e.g., renal tubule epithelial cells, mesangial cells, and podocytes) and infiltrated immune cells (macrophages and T cells) to promote fibrotic progression via inducing apoptosis, ECM protein

synthesis and secretion, and trans-differentiation for de novo myofibroblast generation, a specialized cell type that actively secretes collagens for ECM deposition [24][26][27]. For instance, TGF-β1 induces podocytopenia via apoptosis of podocytes [28], resulting in the progression of glomerulosclerosis [29]. Furthermore, TGF-β1 directly triggers ECM production from renal fibroblasts, mesangial cells, and podocytes [30][31][32] and further generates collagens producing myofibroblasts by trans-differentiating tubular epithelial cells, endothelial cell, and bone marrow-derived macrophages via epithelial-mesenchymal transition (EMT) [33], endothelial-mesenchymal transition (EndoMT) [34], and macrophage–myofibroblast transition (MMT)[24][35] in the fibrotic kidney. TGF-β1 in the diseased kidney activated these processes to accelerate fibrotic progression dramatically.

Smad3 is the canonical downstream of TGF-β1 signaling, serving as a key mediator of kidney fibrosis by promoting myofibroblast accumulation and fibrogenic molecule production in multiple experimental renal diseases, which is dramatically suppressed by Smad3 deletion [36][37][38][39]. Increasing evidence suggests that macrophages are a key player in the Smad3-dependent fibrogenic progression, particularly via the direct mechanism of MMT [40][41]. In a chimeric study with Smad3$^{-/-}$ and Smad3$^{+/+}$ GFP^{+} bone marrow transplanted into irradiated mice with unilateral ureteral obstruction (UUO), Smad3-deleted macrophage (Smad3$^{-/-}$ GFP^{+} F4/80^{+}) failed to undergo MMT to generate myofibroblasts (GFP^{+} α-SMA^{+}) for collagen-I deposition in the fibrotic kidney, which is in contrast to the profound MMT activity of Smad3 wildtype macrophage (Smad3$^{+/+}$ GFP^{+} F4/80^{+}) [42]. These findings demonstrate that Smad3 is the key regulator of MMT [42]. However, targeting Smad3 may cause dysregulation of the immune system, contributing to the development of autoimmune disease [43]. Thus, Smad3 direct downstream targets responsible for pathogenic processes including MMT were identified for developing an antifibrotic strategy with minimal side effects. Single-cell RNA seq resolved the cell-cell transcriptome of MMT, revealing proto-oncogene tyrosine-protein kinase Src- and neural transcription factor Pou4f1-centric regulatory gene networks driving MMT in the injured kidney in vivo and TGF-β1-induced bone marrow-derived macrophages in vitro [24][35]. Further molecular study reveals that Src and Pou4f1 are the direct targets of Smad3, where Src inhibition and macrophage-specific Pou4f1 silencing mimicked the protective effect of Smad3$^{-/-}$ in MMT suppression and associated myofibroblast generation and collagen-I production, representing a precision strategy for targeting MMT [24][35]. Owing to lncRNA's temporal and spatial specificity, Smad3-dependent LncRNA-regulating MMT may be identified to further enhance the precision of targeted MMT antifibrotic therapy.

2.1. TGF-β1-Associated lncRNAs in Kidney Diseases

2.1.1. lncRNAs in TGF-β1 Induced EMT

Studies in recent decades revealed that lncRNA is one of the pathogenic downstream regulators of TGF-β1 signaling in inflammatory diseases [4][18] (**Figure 1**). The majority of lncRNAs exert their biological effects by altering transcriptional or posttranscriptional processes such as transcription factor recruitment, RNA maturation, protein synthesis, and transport. These lncRNAs are also capable of changing chromatin structure via polycomb repressive complex 2 (PRC2) and repressing miRNAs via complementary binding (sponging) [44][45]. For instance, lncRNAs and protein-coding genes were induced via similar mechanisms that shared histone-modification profiles and exon/intron architecture, but lncRNAs are predominantly localized in the nucleus and expressed at a lower level, although with higher tissue specificity compared with coding genes [46]. Most novel lncRNAs were discovered in primates with high-throughput RNA sequencing, revealing disease-associated TGF-β1-dependent lncRNAs that were significantly upregulated in experimental disease conditions in vivo and in vitro [47] (**Table 1**). EMT is a crucial pathogenic process in kidney fibrosis. Cell-cell connections between tubular epithelial cells were progressively lost and ECM molecules were actively produced by EMT-derived cells to transform nephrons into functionless scar tissue [33]. Numerous studies suggested that TGF-β1-dependent lncRNAs regulate EMT. LncRNAs have been proposed to modulate gene expression indirectly as competing endogenous RNAs (ceRNAs), where they compete with a network of mRNAs and circular RNAs (circRNAs) to bind to microRNAs [48]. LncRNAs acting as ceRNAs may represent significant modulation of the canonical TGF-β pathway. PVT1 (plasmacytoma variant translocation 1) is the first lncRNA identified to be associated with diabetic nephropathy, where single-nucleotide polymorphisms (SNPs) significantly associated with end-stage renal disease (ESRD) of type 2 diabetes were located in PVT1 [49]. Further study reveals that high glucose levels induced PVT1 to stimulate TGF-β1, PAI-1, and FN1 expression, which is further amplified by PVT1-derived miR-1207-5p to accelerate ECM accumulation in the diseased glomeruli of diabetic nephropathy (DN) [50]. lncRNA-MGC (megacluster) is a host of 40 miRNAs upregulated in the TGF-β1-treated mesangial cells in vitro and the glomeruli of diabetic mice in vivo via transcription factor CHOP [51]. Inhibiting MGC by antisense oligos GapmeRs in the diabetic kidneys of streptozotocin-injected mice effectively suppressed a cluster of miRNAs and profibrotic gene expression (Col1a2, Col4a1), contributing to the dramatic reduction of PAS-positive areas, glomerular basement membrane (GBM) thickness, and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells [51]. These results suggest a role of miRNAs in the regulation of MGC-driven DN progression.

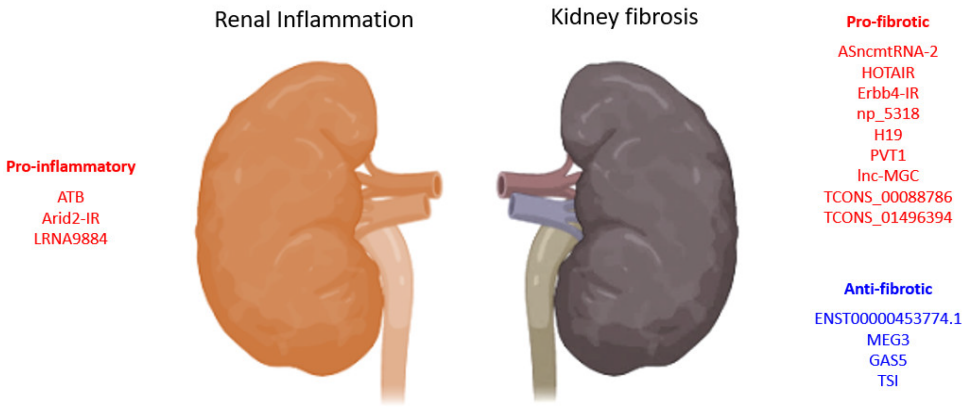

Figure 1. The roles of TGF-β1-dependent lncRNAs in renal disease progression. In the development of chronic renal disease, TGF-β regulates renal inflammation and fibrosis via inducing lncRNAs, which are potential therapeutic targets against CKD development.

In one study, lncRNA-HOTAIR (HOX transcript antisense RNA) was upregulated in UUO kidneys in vivo and TGF-β1-stimulated HK-2 cells in vitro, associated with the Notch signaling (JAG1, Notch1, NICD) and switching of EMT-related proteins, for example, alpha-smooth muscle actin (α-SMA), fibronectin (FN), and E-cadherin [52]. Further analysis shows that HOTAIR sponged miR-124 via a conserved binding site, thus preventing Notch signaling suppression, demonstrated by how the silencing of HOTAIR prevented TGF-β1-induced Notch signaling and EMT, but both were restored by further application of miR-124 inhibitor into the HOTAIR-silenced group [52]. Separately, lncRNA-MEG3 (maternally expressed gene 3) is a protective lncRNA downregulated in TGF-β1-stimulated HK-2 cells, where TGF-β1 suppressed miR-185 to induce CpGs methylation of MEG3 promoter via DNA methyltransferases 1 (DNMT1) [53]. Overexpression of MEG3 largely suppressed TGF-β1-induced apoptosis and EMT [53]. TCONS_00088786 was another pro-fibrotic lncRNA upregulated in the UUO kidney, where TCONS_00088786 silencing effectively suppressed collagen I and III, and profibrotic miR-132 expression [54].

Table 1. The TGF-β-associated lncRNAs in renal diseases.

LncRNA	Biological Process	Model	Species	Mechanism	Year	Ref.
lnc453774.1	anti-fibrosis	HK-2 cells	Human	associated with ceRNAs targeting FBN1, IGF1R, KLF7 PPI networks	2021	[55]
ATB	pro-inflammation	HK-2 cells	Human	promotes apoptosis, senescence, inflammatory cytokines (TNF-α, IL-1β, and IL-6), and adhesion molecules (VCAM-1 and sE-selectin) expression	2020	[56]
HOTAIR	pro-fibrosis	UUO, TECs-HK-2	Human	promotes EMT via Notch1 and miR-124	2019	[52]
ENST00000453774.1	anti-fibrosis	Renal biopsy, UUO, TECs-HK-2	Human	promotes autophagy (Atg5/7) and Nrf2-driven HO-1 expression and suppresses ECM synthesis (Fn, Col-I)	2019	[57]
MEG3	anti-fibrosis	HK-2 cells	Human	suppresses EMT of HK2 cells and is regulated by miR-185/DNMT1/MEG3 pathway	2019	[53]
TCONS_00088786	pro-fibrosis	UUO, NRK52E cells	Rat	promotes collagen I, III, and miR-132 expression	2018	[54]
TCONS_01496394	pro-fibrosis	RNA-seq of rat UUO, NRK52E cells	Rat	promotes Col1a1 and Col3a1 expression promotes Ctgf and Fn1 expression	2017	[58]
ASncmtRNA-2	pro-fibrosis	HRMC, DN	Human, mouse	promotes TGF-β and Fn1 expression	2017	[59]
lnc-MGC	pro-fibrosis	STZ-DN, MMC, MCs	Human, mouse	host of miRNA mega-clusters regulating profibrotic genes expression	2016	[51]

LncRNA	Biological Process	Model	Species	Mechanism	Year	Ref.
PVT1	pro-fibrotic	MC, RPTEC, podocytes	Human	PVT1-derived miR-1207-5p-induced TGF-β1, PAI-1, and FN1	2013	[50]
	pro-fibrotic	ESRD-T2D GWAS	Human	23 SNPs associated with ESRD	2007	[49]

ceRNAs: competing endogenous RNAs, FBN1: fibrillin-1, IGF1R: insulin-like growth factor 1 receptor, KLF7: Kruppel-like factor 7, PPI: protein-protein interaction, ATB: activated by transforming growth factor-β, TNF-α: tumor necrosis factor alpha, IL: interleukin, VCAM-1: vascular cell adhesion molecule 1, HOTAIR: HOX transcript antisense RNA, UUO: unilateral ureteral obstruction, EMT: epithelial-mesenchymal transition, ECM: extracellular matrix, Fn: fibronectin, Col: collagen, MEG3: maternally expressed gene 3, Ctgf: connective tissue growth factor, ASncmtRNA-2: antisense mitochondrial non-coding RNA-2, HRMC: human renal mesangial cell, DN: diabetic nephropathy, MGC: megacluster, STZ: streptozotocin, MMC: mouse mesangial cell, MCs: mesangial cells, PVT1: plasmacytoma variant translocation 1, RPTEC: human renal proximal tubule epithelial cells, PAI-1: plasminogen activator inhibitor 1, ESRD: end-stage renal disease, T2D: type 2 diabetes, GWAS: genome-wide association studies, SNPs: single nucleotide polymorphisms.

2.1.2. lncRNAs Associated with Reactive Oxygen Species

Reactive oxygen species (ROS) are another mechanism leading to fibrotic progression. ROS that accumulate during acute kidney injury cause damage to tubular epithelial cells and the release of pro-inflammatory cytokines, and renal inflammation and fibrosis develop if oxidative stress persists [60][61][62]. Antioxidative and autophagy systems were cellular defense mechanisms against oxidative stress by removing ROS and damaged organelles to limit oxidative damage [63]. LncRNA-ATB was found to be highly expressed in TGF-β1-induced HK-2 cells to promote inflammatory cytokines (TNF-α, IL-1β, and IL-6), adhesion molecules (VCAM-1 and sE-selectin), and pro-senescence factor (p53/p21/p16) expression [56]. In contrast, protective lncRNA 74.1 (ENST00000453774.1) was identified from downregulated differentially expressed genes (DEGs) of TGF-β1-treated HK-2 cells, where it is largely suppressed in the fibrotic tissues compared with the normal control in human renal biopsy [57]. Overexpression of LncRNA 74.1 activates Nrf2/HO-1 antioxidant and Atg5/Atg7/LC3 autophagy pathways in TGF-β1-treated cells, contributing to the protective effect of LncRNA 74.1 overexpression against UUO-induced fibrosis in vivo [57]. ROS are also involved in the pathogenesis of DN, causing mesangial matrix expansion and thickening of the glomerular basement membrane. The lncRNA-ASncmtRNA-2 (antisense mitochondrial non-coding RNA-2) was upregulated in DN of Lepr$^{-/-}$(db/db) mice in vivo and high glucose-stimulated mesangial cells in vitro, promoting TGF-β1 and fibronectin expression in a ROS-dependent mechanism, where shRNA-mediated ASncmtRNA-2-silencing and ROS inhibition by NG-nitro-L-Arginine methylester (L-NAME) effectively suppressed DN and high glucose-induced TGF-β1 and fibronectin expression [59]. Yuan et al. recently identified lnc453774.1(ENST00000453774.1) in TGF-β1-stimulated human kidney epithelial cells, revealing a lnc453774.1-centric fibrotic gene network, interacting with 14 competing endogenous miRNAs to control 8 key functional genes for autophagy, oxidative stress, and cell adhesion (FBN1, IGF1R, KLF7, etc.), suggesting a key regulatory role of lnc453774.1 [55].

2.2. Smad3-Dependent lncRNAs in Kidney Diseases

Smad3 plays an important role in TGF-β-driven renal inflammation and fibrosis, but potential side effects in vital organs limit its therapeutic application [64]. Therefore, Lan's group further identify several Smad3 downstream profibrotic lncRNAs as therapeutic targets against renal fibrosis via RNA sequencing [65] (**Table 2**). Smad3-WT-specific upregulated lncRNAs were extracted from the Smad3-dependent transcriptomes of both UUO and anti-GBM kidneys, eventually revealing 21 potential fibrogenic lncRNAs suppressed by Smad3 deletion [65]. The lncRNAs GAS5 [66], LRNA9884 [67], and lnc-TSI [68] are Smad3 direct targets, regulated by its direct binding to the regulatory sequence of lncRNAs as detected by ChIP-PCR assay, and its influence on the expression levels of lncRNAs was further confirmed by luciferase reporter assay. Smad3 transcriptionally regulates LRNA9884 in the advanced glycation end product-stimulated embryonic fibroblasts (MEFs) and kidney of diabetic mice (db/db) via direct binding on LRNA9884 promoter, promoting MCP-1-mediated renal inflammation via direct binding to its promoter [67]. Moreover, LRNA9884 is also involved in the pathogenesis of acute kidney injury (AKI), which is highly expressed in the tubular epithelial cells of AKI kidneys, promoting IL-1β-induced inflammatory cytokine production (MCP-1, TNF-α, and IL-6) via transcriptional regulation of macrophage migration inhibitory factor (MIF) to trigger MIF/NF-κB pathway [69]. Therefore, LRNA9884 inhibition might be a potential therapy for DN and AKI.

Table 2. The Smad3-associated lncRNAs in renal diseases.

LncRNA	Biological Process	Model	Species	Mechanism	Year	Ref.
GAS5	anti-fibrosis	Smad3-WT/KO UUO, mTECs, MEFs	Mouse	suppresses TGF-β1-induced Col-I/Fn expression and apoptosis, promotes miR-142-5p expression	2021	[66]
LRNA9884	pro-inflammation	Cisplatin-AKI, mTECs	Mouse	promotes IL-1β-induced p-p65,TNF-α, MCP-1, and IL-6, binds directly to MIF promoter	2020	[69]
		Smad3-WT/KO-DN, mTECs, SV40 MES 13	Mouse	Smad3 dependently induced, suppresses IL-1β, TNF-α, and MCP-1, binds directly to the promoter of MCP-1	2019	[67]
Ptprd-IR (np_4334)	pro-inflammation	mTECs, HEK293T, UUO mice	Human, mouse	Smad3 direct target; promotes inflammatory response and macrophage and T-cell infiltration	2020	[70]
Erbb4-IR (np_5318)	pro-fibrotic	Smad3-WT/KO-DN, TECs, MCs	Mouse	Smad3 deletion suppressed Erbb4-IR and restored miR-29b expression	2020	[71]
		AKI, PCS-400-012 cells	Human, mouse	promotes I/R-induced renal cell death, further enhances TGF-β1/Smad3 signaling	2020	[72]
		UUO, TEC, MEF	Mouse	suppresses Smad7 via promoter binding, enhances Smad3-driven Col-I α-SMA expression	2018	[73]
		Smad3-WT/KO-DN, TECs, MCs, MEF	Mouse	enhances Smad3-driven Col-I/IV expression, suppress protective miR-29b via 3'UTR binding	2018	[74]
TSI	anti-fibrosis	UUO, HK2, TECs, MC, HL-7702, LX-2, IMR-90, 16HBE, HKC8 cells	Human, mouse	inhibits Smad3 by direct binding to MH2 domain	2018	[68]
Arid2-IR	pro-inflammation	UUO, TEC	Mouse	Smad3 direct target; promote fibrotic and inflammatory response, macrophage and T-cell infiltration	2015	[75]
RNA-seq	pro-fibrotic	UUO /anti-GBM GN of Smad3-WT/KO mice	Mouse	21 TGF-β/Smad3 dependent lncRNAs	2014	[65]

GAS5: growth arrest-specific 5, UUO: unilateral ureteral obstruction, mTECs: mouse renal tubular epithelial cells, MEFs: mouse embryonic fibroblasts, Col: collagen, Fn: fibronectin, AKI: acute kidney injury, DN: diabetic nephropathy, IL: interleukin, p-p65: phosphorylated p65, TNF-α: tumor necrosis factor alpha, MIF: macrophage migration inhibitory factor, Ptprd-IR: intron of protein tyrosine phosphatase receptor delta, Erbb4-IR: intron of Erb-B2 Receptor tyrosine kinase 4, I/R: ischemia-reperfusion, α-SMA: alpha-smooth muscle actin, UTR: untranslated region, TSI: TGF-β/Smad3-interacting, anti-GBM GN: anti-glomerular basement membranous glomerulonephritis.

LncRNA Ptprd-IR (np_4334, intron of protein tyrosine phosphatase receptor delta), is one of the 21 Smad3-WT-specific upregulated lncRNAs under fibrotic conditions [65]. Smad3 transcriptionally regulates Ptprd-IR expression in TGF-β1-stimulated mouse renal tubular epithelial cells (mTECs) and UUO kidneys via a conserved Smad3 binding site on Ptprd-IR's promoter [70]. Interestingly, Ptprd-IR promotes TGF-β1- and IL-1β-mediated activation of the NF-κB pathway, resulting in pro-inflammatory cytokine production and renal inflammation in UUO kidneys in vivo and mTEC in vitro, while it has no effect on TGF-β1-induced renal fibrosis [70].

LncRNA Erbb4-IR (intron of Erb-B2 receptor tyrosine kinase 4) is expressed in diabetic kidneys of db/db mice, and AGEs stimulated MEFs via a Smad3-dependent mechanism [65][71]. Erbb4-IR promotes fibrotic progression of a diabetic kidney in vivo, and Col-I/IV expression in AGE-stimulated mouse mesangial cells and tubular epithelial cells in vitro, via sponging renal protective miR-29b through direct binding to its 3' untranslated region (UTR) [74]. In a UUO kidney and its in vitro model, Smad3 regulates Erbb4-IR to promote Col-I and α-SMA expression via suppressing Smad7, a suppressor of TGF-β/Smad3 signaling by direct interaction with the 3' UTR of Smad7. Therefore, renal Erbb4-IR silencing effectively restored Smad7 expression against UUO-induced fibrotic progression [74]. This also applied to the pathogenesis of ischemia-reperfusion-induced AKI, where TGF-β/Smad3 signaling was further amplified by Erbb4-IR [72].

Smad3 transcriptionally regulates lncRNA Arid2-IR (np_28496 [75]) expression in UUO kidneys and TGF-β-induced mTEC via direct binding on the promoter region of Arid2-IR. Interestingly, Arid2-IR promotes inflammatory response instead of fibrosis, where NF-κB-driven inflammatory cytokine expression was largely suppressed by Arid2-IR silencing [75], confirming Arid2-IR as a Smad3-associated lncRNA that promotes renal inflammation via crosstalk with the NF-κB pathway [75]. Moreover, LncRNA-TSI directly binds to the MH2 domain of Smad3 to prevent its phosphorylation by TGF-β1 receptor I, thus suppressing Smad3-dependent profibrotic signaling [68]. In addition, J. Sun et al. also revealed 24 upregulated lncRNA candidates by transcriptome analysis of UUO- and Sham-operated renal tissues in which 2 lncRNAs, TCONS_00088786 and TCONS_01496394, contain 4 conserved Smad3 binding motifs and are detected in TGF-β1-stimulated renal tubular epithelial NRK-52E cells [58]. Their pathogenic role in renal fibrosis was confirmed by gene silencing, where TGF-β–induced expression of profibrotic molecules Col1a1 and Col3a1 was regulated by TCONS_00088786, while Ctgf and Fn1 were controlled by TCONS_01496394 [58]. In addition, Smad3-dependent antifibrotic lncRNA GAS5 (growth arrest-specific 5) was largely suppressed in a UUO kidney and TGF-β1-induced mTEC, contributing to the Col-I and Fn expression. Further mechanistic study reveals that GAS5 interacted with miR-142-5p, which binds to the 3′UTR of Smad3 to suppress TGF-β1-induced apoptosis and Col-I/Fn expression of mTEC [66].

3. Therapeutic Strategies Targeting lncRNAs

Due to the dual roles of TGF-β1 in physiological and pathological contexts, targeting TGF-β1 is not an optimal therapeutic strategy as there is evidence that TGF-β1 deficiency might impair host immunity and cause autoimmune diseases [19]. Although many studies have demonstrated that blocking TGF-β1 protects against progressive renal fibrosis and cancer, others have also highlighted the potential consequences of TGF-β1 inhibition, for instance lethal inflammation observed in TGF-β1-deficient mice at 3 weeks of age [76]. Currently, TGF-β1 inhibitors have not been approved for cancer or fibrosis therapy due to reported cytotoxicity in recent clinical trials. A case in point is fresolimumab, a human anti-TGF-β1 monoclonal antibody (mAb), was found to have no significant effect on proteinuria, eGFR, or serum creatinine in focal and segmental glomerulosclerosis (FSGS) [77]. Furthermore, side effects including pustular rash, herpes zoster, bleeding, skin lesions, and cancer have also been observed after anti-TGF-β therapies. Of note, fresolimumab was found to be involved in the development of cutaneous lesions, and results showed that keratoacanthomas were the most common cutaneous neoplasms observed as adverse events in therapies targeting TGF-β [78]. Therefore, targeting the downstream effector of TGF-β may represent a therapeutic strategy specifically against the pathogenic effects of TGF-β without major disturbances to the immune system. Much insight has been gained into how lncRNAs regulate processes such as fibrosis, tumorigenesis, and ECM accumulation, where they can act via binding to Smad proteins, serving as miRNA sponges or interacting with other signaling pathways. These lncRNAs can be used for diagnosis and targeted when their pathogenic mechanisms are elucidated. Findings from preclinical studies have shown the potential of targeting TGF-β-associated lncRNAs for treating kidney diseases and cancers.

Smad3-dependent lncRNAs with therapeutic potential in renal diseases have been identified in previous studies. Inhibition of Erbb4-IR alleviated renal fibrosis in fibrotic UUO and DN models [73][74][79]. Inhibition of Arid2, LncRNA_5318, and LRNA9884 also suppressed renal inflammation in UUO and diabetic models [67][69][75][80]. For antineoplastic therapy, the modulation of lncRNAs not only regulates the TME but also participates in combination with first-line therapy. Gemcitabine and cisplatin are standard therapy for advanced/metastatic carcinoma, where LINC01714 dramatically enhanced the gemcitabine sensitivity of cholangiocarcinoma cells [81]. LncRNA can be targeted by antisense-based strategies or by shRNAs, consisting of siRNAs and modified antisense oligonucleotides (ASOs) [82]. The ASO-based technologies including novel chemical modifications were optimized with multiple preclinical trials, and the efficiency of cellular uptake and the expression levels of targeted ncRNAs have largely improved [83]. The inhibition efficiency and toxicity were major concerns of directly administering lncRNA-targeting agents via tissue or tail vein injection, where toxicity is observed in a dose-dependent manner, i.e., off-target effects through nonspecific binding to similar nucleotide sequences [84][85][86]. However, repeated high dosages of siRNAs and gapmers are required for effective lncRNA inhibition in vivo. Therefore, post-delivery monitoring and optimizing effective concentration of lncRNAs therapeutics are critical for translational application. Novel noninvasive ultrasound microbubble-assisted (USMB) delivery largely reduced the concentrations of lncRNA-targeting agents in nontargeted tissue [87], contributing to the safety and effectiveness in preclinical studies [67][74][88]. Thus, USMB represents a realistic approach to translating lncRNA-targeted therapeutics with added value in postdelivery monitoring and assessment with its imaging function. Moreover, among the FDA- (Food and Drug Administration) and EMA- (European Medicines Agency) approved ASO-based therapies targeting mRNA expression in the liver, most were administered subcutaneously (mipomersen, inotersen, givosiran, volanesorsen, inclisiran, and lumasiran) [5], suggesting the potential for developing subcutaneously delivered lncRNA-targeted ASOs for kidney fibrosis and cancer.

Collectively, lncRNA-targeted therapy might represent an effective strategy for fibrosis and cancer due to its superior tissue and disease specificity. However, the translation of lncRNA-targeted therapy was limited by the dose-dependent toxicities associated with the delivery of lncRNA therapeutics and the lack of conservation among species; that is, human lncRNAs may lack mouse homologs for preclinical study, and mouse lncRNAs may lack human homologs for therapeutic development [89]. Therefore, an effective approach in identifying homologues among species or using humanized mouse models may facilitate the translation of experimental findings into preclinical settings for lncRNA-based therapeutics development [90]. In addition, TGF-β-dependent lncRNAs are disease specific, and in circulation, these lncRNAs are biomarkers of associated diseases. The FDA approved the use of the lncRNA PCA3 in urine as a biomarker for detecting prostate cancer with high sensitivity [91]. As the circulating levels of the TGF-β-associated lncRNAs UCA1 [92], H19 [93], and MALAT1 [94] are associated with disease progression, the potential of these lncRNAs for use as biomarkers for diagnosis could be further explored.

4. Perspectives

The translational development of therapeutics targeting the TGF-β1 signaling pathway has been largely hindered by its key regulatory roles in multiple physiological processes. In recent decades, the dissection of TGF-β1 signaling pathways has revealed numerous precise therapeutic targets, including lncRNAs for inflammatory diseases. Emerging evidence shows that lncRNAs are specific pathogenic mediators of TGF-β1, regulating a particular function of TGF-β1 during inflammatory disease progression that can be targeted to develop effective gene-based therapies. With the advancement of RNA sequencing at single-cell resolution and bioinformatic analysis, a more in-depth regulatory mechanism of lncRNAs in inflammatory diseases will be discovered. Disease- and cell-type-specific lncRNAs will be identified for the development of precision therapies against tissue inflammation and cancers.

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