Non-coding RNA Research 2 (2017) 68–73

Contents lists available at ScienceDirect

Non-coding RNA Research

journal homepage: http://www.keaipublishing.com/NCRNA

TGF-β1 signaling in kidney disease: From Smads to long non-coding RNAs

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ARTICLE INFO

Article history:
Received 28 December 2016
Received in revised form
14 March 2017
Accepted 6 April 2017
Available online 8 April 2017

ABSTRACT

Transforming growth factor-β1 (TGF-β1) has an essential role in the development of kidney diseases. However, targeting TGF-β1 is not a good strategy for fibrotic diseases due to its multifunctional characteristic in physiology. A precise therapeutic target maybe identified by further resolving the underlying TGF-β1 driven mechanisms in renal inflammation and fibrosis. Smad signaling is uncovered as a key pathway of TGF-β1-mediated renal injury, where Smad3 is hyper-activated but Smad7 is suppressed. Mechanistic studies revealed that TGF-β1/Smad3 is capable of promoting renal inflammation and fibrosis via regulating non-coding RNAs. More importantly, involvement of disease- and tissue-specific TGF-β1-dependent long non-coding RNAs (lncRNA) have been recently recognized in a number of kidney diseases. In this review, current understanding of TGF-β1 driven lncRNAs in the pathogenesis of kidney injury, diabetic nephropathy and renal cell carcinoma will be intensively discussed.

1. Introduction

Transforming growth factor-β (TGF-β) plays an important role in both pathological and physiological processes. The pathological roles of TGF-β in tissue fibrosis, inflammation, diabetic complication, tumor growth and metastasis have been extensively studied. Its signaling event starts from the protease cleavage of latent TGF-β to form active TGF-β which then binds and activates TGF-β receptor 2 (TGFR2) thereby triggering recruitment and activation of TGF-β receptor 1. As a result, phosphorylated Smad2 and Smad3 bound to Smad4 as a complex and translocate into the nucleus. This Smad2/3/4 complex binds to the regulatory regions of target genes (protein coding or non-coding) and mediates their transcriptions in order to promote the pathogenesis specific for a disease [1].

TGF-β1/Smad signaling pathway is dramatically activated in experimental animal models and human kidney diseases [2]. TGF-β1 activates Smad3 to contribute to fibrosis; whereas overexpression of Smad7 prevents renal fibrosis and inflammation *in vitro* and *in vivo* [3–6]. Although Smad3 is a key transcription factor in response to many pathogenic mediators, systemic Smad3 knockout mice shows that targeting Smad3 may cause autoimmune disease by impairing immunity [7]. Thus, alternative approaches to inhibit TGF-β/Smad-mediated actions specific to disease development should be targeting on the downstream effector genes of the TGF-β/Smad3 signaling.

Emerging evidence shows that non-coding RNAs (ncRNAs) play an important role in the development of kidney diseases [8–10]. Targeting disease-associated miRNAs was supposed to be an ideal therapeutic strategy for kidney disease [11,12]. Unfortunately, off-target effects and low specificity of miRNAs are recently noticed, which let scientists reconsider the development of miRNA-targeted therapy [13]. This is because miRNAs act as cofactor rather than function-specific gene in biology, their regulatory and working mechanism are multifactorial and complicated. In contrast, important roles of long noncoding RNA (lncRNA), transcript longer than 200 nucleotides lacking protein-coding capacity, in the pathophysiological progression of tumors, autoimmune diseases, and cardiovascular diseases have been recently recognized since the discovery of a lncRNA required for mammalian X chromosome

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http://dx.doi.org/10.1016/j.ncrna.2017.04.001

inactivation (Xist) [14–19]. However, lncRNAs are gaining more attention as an emerging therapeutic target, as they are capable to regulate multiple genes expression in a disease- and tissue-specific manner [20,21]. This review focuses on how TGF-β1/Smad3-dependent lncRNAs involve in kidney diseases, specifically on kidney injury, diabetic nephropathy as well as renal cell carcinoma.

2. Smad3-dependent lncRNAs in kidney injury

Renal inflammation and fibrosis are hallmark and common pathways leading to end-stage organ diseases, including acute kidney injury (AKI) and chronic kidney disease (CKD). Renal inflammation is an initial response to the kidney injury and is a key process leading to CKD [22,23]. By using RNA-sequencing (RNA-seq), we identified a number of Smad3-dependent lncRNAs participate in renal fibrogenesis of mice with kidney injury induced by unilateral ureteral obstruction (UUO) and anti-glomerular basement membrane glomerulonephritis (*anti*-GBM GN) models [24]. Compared with the wild-type mice, 21 novel Smad3-depedent lncRNAs including np_5318 and np_17856 were identified in both of the renal inflammation and fibrosis in a Smad3-dependent manner *in vivo* [24]. Lan's group further characterized Arid2-IR, one of the Smad3-dependent lncRNAs, and eventually revealed its specific functional role in renal inflammation [25]. A Smad3 binding site was identified in the promoter region of Arid2-IR which triggers its transcription in the diseased kidney. Interestingly, kidney-specific knockdown of Arid2-IR blunted NF-κB-driven renal inflammation without affecting TGF-β1/Smad3-mediated renal fibrosis in the obstructed kidney *in vivo*, showing by ultrasound-microbubble-mediated gene transfer technique [25]. The study discovered that silencing of Arid2-IR in UUO kidney significantly reduced inflammatory cells infiltration ($F4/80^+$ macrophages and $CD3^+$ T cells), cytokines production (IL-1β, TNF-α, and MCP-1) and NF-κB signaling activation (NF-κB/p65 phosphorylation and nuclear localization) in the UUO kidney. Consistently, transient overexpression of Arid2-IR in murine tubular epithelial cells promoted IL-1β induced NF-κB signaling and inflammatory cytokine production without alternating TGF-β1-induced fibrotic response *in vitro* [25].

In glomerular nephritis, expression level of lncRNA XIST (but not Neat1) was elevated by glomerular podocyte injury and can be detected in human urinary samples. XIST is induced in the membranous nephropathy (MN) kidney, and Xist can be detected in the ascites and urine of mice with MN [26]. Mechanistically, ChIP assay targeting H3K27me3 and immunohistochemistry uncovered that trimethylation was largely reduced at the Xist promoter region in mice with MN [26]. These findings suggested XIST as a potential MN-associated kidney injury biomarker for potential prognostic use in clinic.

In addition, an intronic lncRNA MGAT3-AS1 (TapSAKI) was identified in kidney biopsies and plasma samples from patients with AKI. It is up-regulated in the plasma of patients with AKI comparing with the control group [27]. The plasma level of circulating MGAT3-AS1 correlated with disease severity and specifically up-regulated in the tubular epithelial cells under hypoxia. Furthermore, lncRNA-PRINS, a HIF-1α-responsive lncRNA, was suggested in regulating RANTES production and the progression of AKI [28]. The lncRNA-PRINS may mediate the expression of RANTES (regulated on activation, normal T-cell expressed and secreted), an inflammatory mediator involved in renal inflammation, during AKI in a HIF-1 α-dependent manner [28]. These lncRNAs may serve as the potential biomarker and therapeutic target for AKI.

Nevertheless, Lan's group further revealed two Smad3-dependent lncRNAs np_5318 and np_17856 for renal fibrosis. These lncRNAs were significantly evoked in the kidney of mice with UUO; their expression levels were further enhanced by the knockout of Smad7 but largely repressed by the knockout of Smad3 knockout and overexpression of Smad7 [24]. In addition, they were identified as a direct target gene of Smad3, because TGF-β1 stimulation dramatically enriched Smad3 binding on their promoter regions showing by ChIP assay. The results implied that these two lncRNAs might act as effector molecules at the downstream of TGF-β/Smad3 signaling. Thus, further investigation and characterization should be conducted to identify their pathogenic roles and working mechanisms in kidney fibrosis. (See Table 1).

3. LncRNAs mediate TGF-β1 signaling in diabetic nephropathy

Diabetes mellitus is the leading cause of end-stage renal disease (ESRD) in diabetic patients. PVT1 is the first identified lncRNA associated with ESRD in type 1 and type 2 diabetes [29,30]. Using genome-wide single nucleotide polymorphism association study, its association between ESRD and diabetes was first revealed by Hanson et al. where several SNPs (especially rs2648875) with a strong association with ESRD were located within PVT1 gene [29]. Using similar approach, the association between SNP (rs13447075) in PVT1 transcript variant 6 and ESRD was further suggested by Millis et al., the study also demonstrated that expression level of PVT1 transcript variant 6 was higher than other variants in human renal cells [30]. The function of PVT1 then further characterized in diabetic kidney disease by Alvarez and DiStefano: high glucose condition induced the expression of PVT1, FN1, and COL4A1 and the secretion of TGF-β1, PAI-1, FN1, and COL4A1 in human mesangial cells (MC), but the secretion of FN1 and COL4A1 were repressed by PVT1 knockdown indicating the pathological role of PVT1 and its involvement in TGF-β1 mediated diabetic nephropathy [31]. The role of TGF-β signaling in the working mechanism of PVT1 was further investigated by Alvarez et al. Expression of miR-1207-5p is higher than other PVT1 derived miRNAs in human renal proximal tubule epithelial cells, podocytes, and mesangial cells, and is up-regulated by high glucose condition and TGF-β stimulation independent to PVT1 [32]. Under high glucose condition, expressions of miR-1207-5p and TGF-β1 (time- and dose-dependent) were induced, while PMEPA1, PDPK1, and SMAD7 were repressed; PVT1 miR-1207-5p acts consistent with Pvt1 to trigger secretion of TGF-β1, PAI-1, and FN1 in the primary murine MC *in vitro* [32].

In addition, lncRNA MALAT1 is increased in diabetic kidney and regulates hyperglycemia-induced renal inflammation specifically in endothelial cells, and alters H19 expression in the kidneys of embryos carried by hyperglycemic mothers [33–35]. In STZ-induced type 1 diabetic mouse model, reduction of Miat in renal tubule associated with high serum creatinine and blood urea nitrogen of the diseased mice [36]. The relationship between MIAT and kidney injury was further investigated in cultured cell model, high glucose condition significantly reduced the viability, expression of MIAT and NRF2, and translocation of nuclear NRF2 in the human renal tubular epithelial cell HK-2 [36]. In addition, overexpression of MIAT restored the high glucose-mediated anti-proliferation and NRF2 reduction in HK-2 cells suggesting the protective role of MIAT in diabetic nephropathy [36].

Furthermore, lncRNA CYP4B1-PS1-001 regulates proliferation and fibrosis of mesangial cells in diabetic nephropathy. It was particularly down-regulated in response to early diabetic nephropathy in db/db mice *in vivo*. CYP4B1-PS1-001 specifically expressed in murine mesangial cells instead of human proximal tubular epithelial cell line HK-2 nor baby hamster Syrian kidney cell line BHK-21 *in vitro*, while its overexpression was able to inhibit the proliferation and fibrosis of mesangial cells under high glucose condition *in vitro* [37]. Interestingly, a protective lncRNA ENSMUST00000147869 is also reported by Wang et al.;

Table 1
LncRNAs in kidney injury.

lncRNA	Model	Expression level	Mechanism	Reference
Arid2-IR	UUO, mTECs	Up	NFκB signaling	[25]
XIST/Xist	cBSA induced MN Nephropathy biopsy E11 podocytes	Up	Reduce trimethylation at Xist promoter	[26]
MGAT3-AS1 (TapSAKI)	AKI patient Tubular epithelial cells	Up	Hypoxia	[27]
PRINS	AKI patient IRI kidney	Up	Interacts with RANTES	[28]

overexpression of ENSMUST00000147869 protects mesangial cells against high glucose induced proliferation and fibrosis [39]. (See Table 2).

4. TGF-β1-dependent lncRNAs regulates development of renal cell carcinoma

TGF-β1 has been reported to play a suppressive role in carcinogenesis, but ironically tumor cells also produce TGF-β1 to support their development [39–42]. In addition, inflammation is a curial step for not only fibrosis but also carcinogenesis, and increasing evidence shows that the progression of cancer is TGF-β1 dependent [43,44].

TGF-β1 induced the expression of Hotair which down-regulating E-cadherin but up-regulating vimentin and beta-catenin in renal carcinoma cells [45]. Silencing of HOTAIR largely inhibited the proliferation and migration of renal carcinoma cell lines due to induction of cell cycle arrest at G0/G1 phase *in vitro*, cell cycle related genes (p53, p21, and p16) were modulated via histone methylation [46]. The role of HOTAIR in tumor was further confirmed in xenograft model *in vivo*, where knockdown of HOTAIR silencing significantly inhibit the human tumor growth rate in mice [46]. In addition, Chiyomaru et al. demonstrated that the protumoral function of HOTAIR can be suppressed by miR-141 via a Ago2-dependent mechanism [47,48].

Another TGF-β1-dependent lncRNA-ATB mediates epithelial–mesenchymal transition (EMT) of carcinoma cells and is associated with invasion and metastasis of hepatocellular carcinoma as well as renal cell carcinoma [49,50]. The expression level of lncRNA-ATB increases in renal tumor tissue compared to normal tissue as well as in human renal cell carcinoma cell lines. More importantly, it is significantly increased in the metastatic patients compared to patients with localized renal cell carcinoma [50]. Silencing of lncRNA-ATB inhibited the proliferation, invasion, and migration of renal carcinoma cells *in vitro*, probably due to the induction of apoptosis and reduction of EMT via vimentin and cadherin switching [50].

Nevertheless, reduction of another lncRNA MEG3 is observed in the biopsies of patients with renal cell carcinoma [51]. Overexpression of MEG3 in renal carcinoma cells inhibites Bcl-2, procaspase-9 but promotes caspase-9 cleavage and cytochrome c *in vitro*, suggesting overexpression of MEG3 induce apoptosis via a mitochondrial-dependent pathway [51]. In addition, MEG3 also regulates genes related to TGF-β1 pathway [52]. In contrast, induction of lncRNA RCCRT1 is found in the human renal cell carcinoma tissues and associated with lower survival after surgery, and silencing of RCCRT1 in renal cell carcinoma cells *in vitro* revealed that RCCRT1 involves in the proliferation, migration, and invasion of the cancer cells [53].

In addition, clear cell renal cell carcinoma (ccRcc) is the most common subtype of kidney cancers, featuring epithelial cells with clear cytoplasm. Several TGF-β1-dependent lncRNAs involve in the pathogenesis of ccRcc. For example, MALAT1 involves in TGF-β1 induced cancer cell metastasis *in vitro* [54]. Zhang et al. and Xiao et al. discovered the association between MALAT1 and ccRcc by using the primary ccRcc patient sample and the Cancer Genome Atlas (TCGA) Data Portal of starBASE v2.0 respectively [55,56]. Overexpression of MALAT1 is found in a number of human renal carcinoma cell lines (786O, ACHN, Caki-1, and Caki-2) compared with normal renal cell line (HK-2), and high expression level of MALAT1 positively correlated to the lower long-term survival rate in patients with ccRcc [56]. Xiao et al. confirmed the pathogenic role of MALAT1 on animal model, silencing of MALAT1 reduced human tumor size *in vivo*. Mechanistically, Zhang et al. found that MALAT1 contributes to the proliferation, migration, invasion of renal cancer cells (786O and ACHN) showing by MTT, cell cycle analysis, wound healing assay, and trans-well invasion assay [56]. MALAT1 acts as competing endogenous RNA to antagonize the

Table 2
LncRNAs mediate TGF-β1 signaling in diabetic nephropathy.

lncRNA	Model	Trend in disease	Mechanism	Reference
PVT1/Pvt1	T1DM, T2DM patients Renal epithelial cells, PTECs, CECs, and mesangial cells	up	induces FN1, COL4A1, TGFβ1, PAI-1	[29–32]
MALAT1/Malat1	STZ-induced DN Rf/6A cells HUVECs	Up	SAA-mediated IL-6 and TNF-α increases ICAM-1, VEGF, TNF-α endothelial cell migration tube formation p38-MAPK mediated proliferation	[33, 35]
MIAT/Miat	STZ-induced DN HK-2 cells	Down	enhances Nrf2 expression	[36]
CYP4B1-PS1-001	db/db mice murine mesangial cells, HEK293T, HK-2, BHK-21	Down	suppresses PCNA, Cyclin D1, Collagen I and fibronectin expression and mesangial cells proliferation	[37]
ENSMUST00000147869	db/db mice HEK293T cells Mouse mesangial cells	Down	suppresses PCNA, Cyclin D1, collagen 1, and fibronectin expression	[38]

action of miR-200s thus regulating the expression of ZEB2 and the cancer cell proliferation, migration, and invasion [55]. Moreover, Hirata et al. further investigated the working mechanism of MALAT1 in renal cell carcinoma that overexpression of c-Fos triggers expression of MALAT1, suggesting C-FOS mediates the MALAT1 at transcriptional level [57]. Interestingly, the activity of MALAT1 maybe mediated by physical binding of EZH2 independent to C-FOS, and western blotting of A-498 and 786-O cells showed that MALAT1-knockdown leads to reduce methylation of E-Cadherin gene promoter (H3K27me3) and expression of EZH2, β-catenin, and C-MYC [57]. Similar to MIR200s, MIR205 acts as a competing endogenous RNA that represses the expression of MALAT1 and vice versa in the cancer cells [57].

Nevertheless, SPRY4-IT1 is another TGF-β1-dependent lncRNA involves in ccRcc. Induction of SPRY4-IT1 was first reported to mediate EMT in esophageal squamous cell carcinoma via regulating vimentin and fibronectin expressions [58]. In ccRcc, SPRY4-IT1 is evoked in the human tumor samples and human renal carcinoma cell lines, patients with high expression level of SPRY4-IT1 in tumor associated with a lower long-term survival rate [59]. Silencing of SPRY4-IT1 significantly inhibited the proliferation, migration, and invasion of the renal carcinoma 786-O cells *in vitro* [59]. (See Table 3).

5. Perspective for lncRNA research in kidney diseases

LncRNA is highly disease- and tissue-specific that makes it becomes an ideal therapeutic target for developing treatment against kidney disease [20,21]. Compared with protein-coding RNAs, lncRNAs are more specific to organs, tissues, cell types, developmental stages, and disease conditions, making them promising candidates as diagnostic and prognostic biomarkers and as gene therapy targets. More than 85% of disease-related SNPs are within noncoding regions and are strikingly overrepresented in enhancer and promoter regions, suggesting the importance of lncRNA loci at these SNP harboring regions to human diseases [20]. Unique features of lncRNA in disease development and regulation eliminated the doubts in development of ncRNA-targeted therapy, as off-targeted effect, avoidance from internal nucleases, and toxicity of miRNA-targeted therapy were observed in a number of studies [13].

Indeed, lncRNA is a new era if biological research therefore related information is still very limited. So far, MALAT1 is one of the most comprehensively studied lncRNAs in kidney diseases. This lncRNA is significantly increased in patients with diabetic nephropathy as well as renal cell carcinoma; the same trend is demonstrated on their respective experimental animal models [33,35,54–57]. MALAT1 expression can be induced by TGF-β1 [54], and Malat1 consistently promotes cell proliferation suggested by all the studies. However its working mechanisms are multifactorial in different disease models. Interestingly, it triggers inflammation and tube formation in diabetic nephropathy [33,35], whereas it induces cancer cell metastasis via MIR-200s suppression [55]. Further investigation should be done to identify a common pathogenic mechanism of MALAT1, in order to develop an effective therapy for kidney diseases.

However, there are reasons seriously limited the development of lncRNA research at this moment. First, an official lncRNA database, which provides reliable and comprehensive updates of information about potential molecular and cellular functions of lncRNAs, is needed in an urgent. Although databases (e.g. Noncode, lncRNAdb, NRED, etc …) frequently update based on new literature, their bioinformation of lncRNAs are not as consistent as traditional sites such as PubMed and EBI [60]. Nevertheless, an effective method to find out lncRNA homologues between different species

Table 3
TGF-β1-dependent lncRNAs in renal cell carcinoma.

lncRNA	Model	Trend in disease	Mechanism	Reference
HOTAIR/ Hotair	MCF10a, HCC1954, DLD1, and HT29 cells	Up	TGF-β1 induced HOTAIR expression	[45]
	BALB/c nude mice Human renal cancer cell lines A-498 and OS-RC-2	Up	induced p53, p21 and p16 expression via H3K27me3	[46]
	786-O, ACHN, DU145, HT-29, and HK-2 cells	Up	regulates ABL2, PCDH10, Snail, and ZEB1 expressions miR-141 inhibits HOTAIR via Ago2	[47]
	769-P, 786-0, and Kert-3	Up	mediates cell migration	[48]
lncRNA-ATB	BALB/c nude mice Human biopsy SMMC-7721, QSG-7701, MCF7, SW480 cell lines	Up	induces cancer cell colonization via IL-11/STAT3 signaling TGF-β1 triggers ATB expression	[49]
	Human RCC biopsy 786-O, A498, ACHN, and HK-2 cells	Up	promotes EMT, migration and invasion, and suppress apoptosis	[50]
MEG3	Human tissue biopsy Human renal cancer cell lines 786-0 and SN12	Down	induces apoptosis via modulating expression of Bcl-2, caspase-9 cleavage, and cytochrome c release.	[51]
	BT-549, MDA-MB-231	N/A	modulates the activity of TGF-β genes	[52]
RCCRT1	Human tissue biopsy ACHN and A498 cells	Up	promotes proliferation, migration, and invasion of cancer cells	[53]
MALAT1/ Malat1	BALB/C nude mice Human bladder cancer cells Human bladder biopsy	Up	TGF-β1 induced expression of MALAT1	[54]
	Human tissue biopsy BALB/C nude mice ACHN, 786-O, SN12-PM6, HK-2, CAKI-1, and OS-RC-2 cells	Up	induces proliferation and metastasis of cancer cell inhibit MIR-200s to induce ZEB2 expression	[55]
	Human tissue biopsy HK-2, 786-O, ACHN, Caki-1, and Caki-2 cells	Up	promotes cell proliferation, cell migration, invasion and reduces G0/G1 proportion	[56]
	Human tissue biopsy Human renal cancer cell lines 786-O, A-498, Caki-2, and Caki-1	Up	enhance Ezh2, β-catenin, and c-Myc expression	[57]
SPRY4-IT1	Eca109, KYSE150, Eca9706, EC18, EC1, and HEEC cells	Up	mediates TGF-β1 induced expression changes in E-Cadherin, Vimentin, and Fibronectin	[58]
	Human tissue biopsy 786-O, ACHN, Caki-1, Caki-2, and HK-2 cells	Up	promotes cell proliferation, migration, invasion and reduces G0/G1 proportion	[59]

is in an urgent needed. Indeed, a number of lncRNAs identified from animal disease models cannot translate into human and vase verse. Evolutionary conservation has been a confusing and challenging area of lncRNA research, as most lncRNAs are not fully conserved across mammals [61]. It is suggested that lncRNAs have functional orthologs, i.e. genes with similar function but no ancestral relationship, supported by the case of functional orthology between lncRNAs XIST and RSX [62]. Functional orthology cannot be studied with computational methods; an appropriated strategy should be developed for overcoming this barrier.

6. Conclusion

Increasing evidence shows that lncRNA is not only an association with disease processes, but also a pathogenic mediator involves in the development of kidney diseases. The tissue- and disease-specific characteristic of lncRNAs makes it to be a potential biomarker as well as therapeutic target for clinical settings, although their underlying working mechanisms are still largely unknown. Thus, identification and characterization of kidney disease associated lncRNAs may represent a promising research strategy for resolving renal disorder and may lead to the development of precision therapies for kidney diseases.

Statement of competing financial interests

None.

Acknowledgments

This work is supported by grants from the Research Grants Council of Hong Kong (GRF 14117815, CUHK3/CRF/12R, T12-402/13N and the Major State Basic Research Development Program of China (2012CB517705)

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