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Long Noncoding RNAs and Their Therapeutic Promise in Diabetic Nephropathy

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Abstract
Recent advances in large-scale RNA sequencing and genome-wide profiling projects have unraveled a heterogeneous group of RNAs, collectively known as long noncoding RNAs (lncRNAs), which play central roles in many diverse biological processes. Importantly, an association between aberrant expression of lncRNAs and diverse human pathologies has been reported, including in a variety of kidney diseases. These observations have raised the possibility that lncRNAs may represent unexploited potential therapeutic targets for kidney diseases. Several important questions regarding the functionality of lncRNAs and their impact in kidney diseases, however, remain to be carefully addressed. Here, we provide an overview of the main functions and mechanisms of actions of lncRNAs, and their promise as therapeutic targets in kidney diseases, emphasizing on the role of some of the best-characterized lncRNAs implicated in the pathogenesis and progression of diabetic nephropathy.

Introduction

The central dogma in molecular biology describes RNA molecules only as intermediates by which the information in genes flows from DNA to proteins. However, according to an RNA-centric view of the universe, RNA preceded DNA molecules in evolution and was the primitive form of storing genetic information in organisms. This is particularly of relevance when it was noted that indeed only ~2% of the roughly 3 billion base pairs of DNA in the human genome represent protein-coding sequence, while the remaining ~98% are composed of noncoding RNAs (ncRNAs) [1–3]. ncRNAs are classified accordingly to their size and functions. More abundant ncRNAs include ribosomal RNA; transfer RNA, which are directly related to protein synthesis; small interfering RNA; small nuclear RNA; small nucleolar RNA; PIWI-interacting RNA; microRNAs (miRNAs); circular RNAs (circRNAs); and long ncRNAs (lncRNAs) [4–7] (Table 1).

Among ncRNAs, lncRNAs have recently attracted intense attention because of a growing body of evidence suggesting their involvement in a variety of physiological and pathological pathways. They are classically defined as

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Table 1. Classification of ncRNAs

Name	Size
rRNA	~1.9 kb
tRNA	~76–90 nt
siRNA	~21 nt
snRNA	~100–300 nt
snoRNA	~60–300 nt
piRNA	~26–30 nt
miRNA	~18–21 nt
circRNA	~246–467 nt
lncRNA	>200 nt

rRNA, ribosome RNA; tRNA, transfer RNA; siRNA, small interfering RNA; snRNA, small nuclear RNA; snoRNA, small nucleolar RNA; piRNA, PIWI-interacting RNA; miRNA, microRNA; circRNA, circular RNA; lncRNA, long noncoding RNA; ncRNA, noncoding RNA.

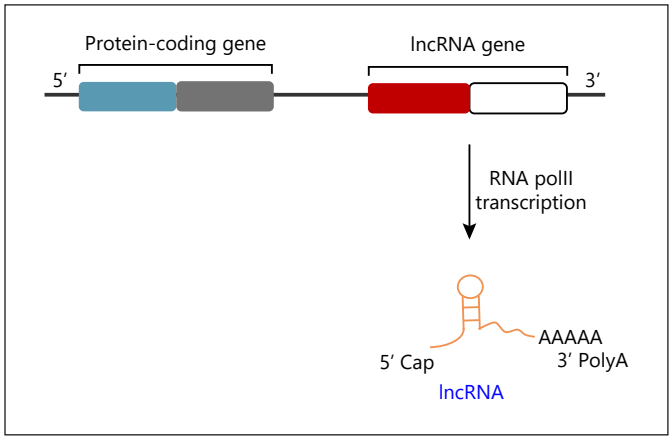

Fig. 1. Transcription of lncRNA genes. Like protein-coding genes, a majority of lncRNA genes are transcribed by RNA Pol II, processed to gain 5′ Cap and 3′ PolyAs to generate functional lncRNAs. Pol II, polymerase II; lncRNA, long noncoding RNA.

RNA molecules that are at least 200 nucleotides in length and do not exhibit a potential to encode for functional proteins [1, 5, 8]. Notably, lncRNAs outnumber protein coding transcripts due to advances in RNA sequencing (RNA-seq). Based on the Encyclopedia of DNA Elements Project Consortium (GENCODE 35), 16,899 lncRNAs are identified in the human genome with 46,977 distinct lncRNA transcripts. Most of lncRNAs are similar to normal coding mRNAs in that they are generally transcribed by RNA polymerase II, 5′ capped, spliced and polyadenylated at their 3′ ends (Fig. 1). Furthermore, the majority of lncRNAs present similar epigenetic marks at their promoter regions such as the increased occurrence of trimethylation of lysine 4 of histone 3 (H3K4me3) and the presence of RNA polymerase II (Pol II) binding sites, which serves as an indicator of active transcription (http://www.lncrnadb.org) [9]. However, lncRNAs are generally expressed at lower levels and are evolutionarily less conserved than mRNAs [10, 11]. One important caveat is the ability of lncRNAs to bend and create secondary and tertiary structures, and indeed it has been suggested that functional domains of lncRNAs formed upon folding could be more evolutionarily conserved, instead of their sequences [7, 9, 12, 13]. In addition, lncRNA promoters are known to be more conserved than lncRNA exons [14]. Another key characteristic of lncRNA, in contrast to coding RNA, is that lncRNAs are more cell- and tissue-specific and temporally regulated, features that make their discovery more complicated. According to their relative locations with respect to protein-coding genes, lncRNAs can be broadly classified into sense, antisense, bidirectional, and intergenic based on the relationship between lncRNA and its neighborhood gene (Fig. 2). Of note, following the development of new forms of lncRNA, the traditional classification of lncRNA is constantly under revision taking additional criteria into account, including their molecular function and their splicing modes.

lncRNAs and Their Divergent Mechanism of Action

Despite all the recent progress in lncRNA biology and their major roles in a vast array of biological activities in the cell, much of scientific community is still raising doubts on whether the majority of lncRNAs are functional. The main arguments made against the functional importance of lncRNAs are their low levels of expression and sequence conservation [1, 15]. However, several recent studies counter the assumptions of these arguments by clearly providing strong evidence on the critical roles of lncRNAs in cell cycle regulation, pluripotency, imprinting genomic loci, chromatin remodeling, and telomerase length, just to name a few [13, 16]. Furthermore, the direct correlation between lncRNA numbers and the complexity of the organisms suggest a high degree of functionality [2, 3].

lncRNAs can activate or repress genes by several mechanisms at epigenetic, transcriptional, posttranscriptional, and translational levels. A major cellular function of lncRNAs is related to their epigenetic effect to influence chromatin remodeling by recruiting or preventing the interaction of histone or chromatin modifiers to spe-

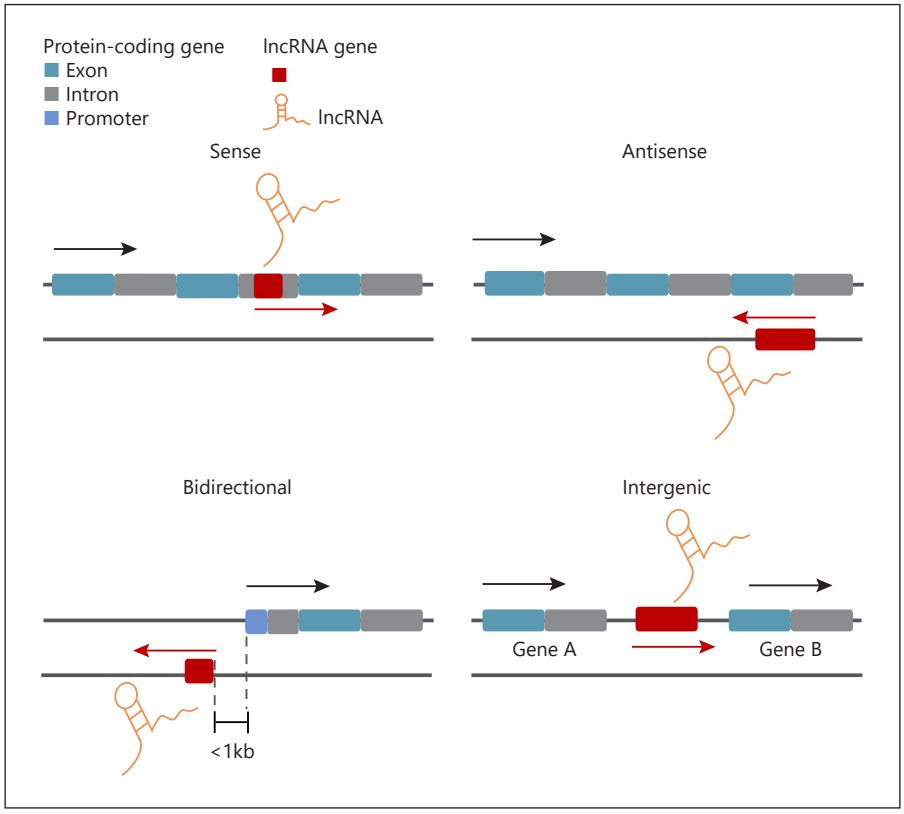

Fig. 2. Classification of lncRNA based on their genomic locations. With respect to orientation and genomic position of protein-coding genes, lncRNAs are classified into 4 major categories. (1) Sense lncRNAs that are transcribed from the sense strand, within an intronic region of protein-coding genes; (2) antisense lncRNAs that are transcribed from the opposite strand of protein-coding genes; (3) bidirectional lncRNAs that are transcribed from the opposite strands, in the opposite direction and within 1 kb of the promoter of protein-coding genes; and (4) intergenic lncRNAs that are transcribed in the genomic region between 2 protein-coding genes (genes A and B). lncRNA, long noncoding RNA.

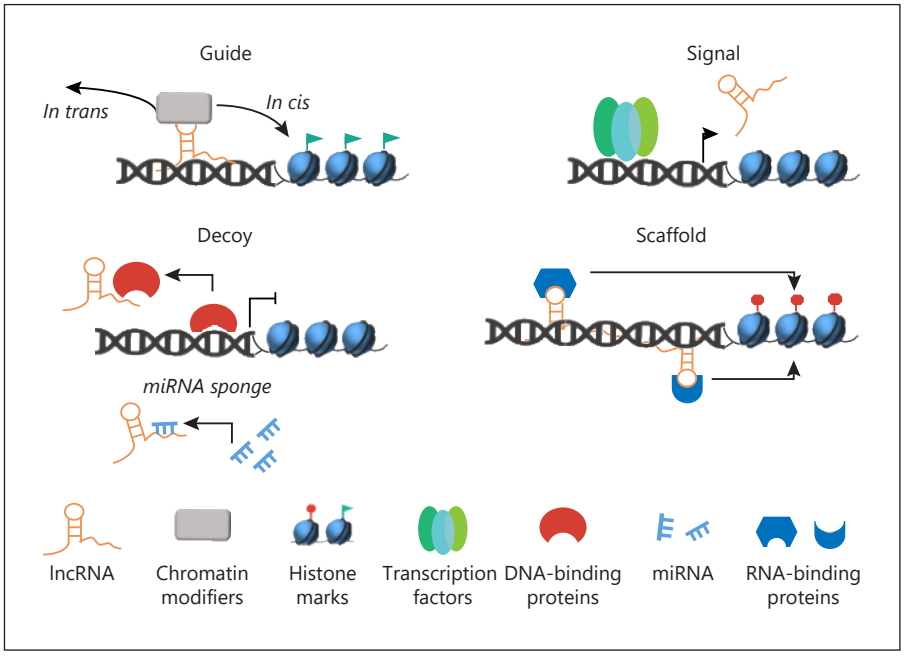

Fig. 3. Diverse mechanisms of action of lncRNA. lncRNAs could serve as (1) guides: lncRNAs may act as guides to recruit proteins to regulate the expression of other genes. (2) Signals: lncRNAs can serve as molecular signals where their expressions are regulated by diverse stimuli at specific time and place. (3) Decoys: lncRNAs can act as decoys that titrate away DNA-binding proteins, or regulatory RNAs (e.g., miRNAs). (4) Scaffolds: lncRNAs may act as scaffolds to bring 2 or more effector molecules (proteins and/or DNAs) to assemble into RNP complexes. lncRNA, long noncoding RNA; miRNA, microRNA; RNP, ribonucleoprotein.

cific DNA loci [14, 17–20]. For example, lncRNAs can recruit polycomb repressive complexes or histone methyltransferases to regulate DNA accessibility through histone modification [14, 19, 20]. A growing body of evidence has identified the active role of lncRNAs on transcription by interacting with transcription factors, or any other proteins involved in gene expression, leading to transcriptional regulation of target genes. lncRNAs can

Table 2. lncRNAs in DN [46, 51, 54, 56–58, 61, 64, 69–71, 74, 76, 91–101]

Mechanism of action	lncRNA	Source
Decoy	Erbb4-IR	[51]
	MEG3	[54, 56, 57]
	HOTAIR	[91]
	GAS5	[58]
	XIST	[61]
	Gm6135	[92]
	H2k2	[93]
Signal	ZEB1-AS1	[94]
Scaffold	lnc-MGC	[64]
Guide	CYP4B1-PS1-001	[95, 96]
	ENSMUST00000147869	[97]
	NONHSAG053901	[98]
	TUG-1	[46, 69, 70]
	Rpph1	[99]
	LRNA9884	[100]
	MALAT1	[71]
	NEAT1	[74, 76]
	SPAG5-AS1	[101]

lncRNA, long noncoding RNA; DN, diabetic nephropathy.

also be involved in multiple posttranscriptional modifications, including mRNA stability, splicing and nuclear export, and protein translation processes [21, 22]. Functionally, lncRNAs can be subdivided into several main categories based on their molecular functions (Fig. 3): (1) guide lncRNAs, when they recruit chromatin-modifying enzymes to modulate the expression of local genes (in cis) or target genes in other chromosome (in trans); (2) signal lncRNAs, serving as molecular signals where their expression is regulated by transcription factors and responding to diverse stimuli (e.g., cold and DNA damage) in a time and space dependent manner; (3) decoy lncRNAs, whereby lncRNAs titrate away DNA-binding proteins (e.g., transcription factors), modulating their regulation. Decoy lncRNAs could also serve as sponges for regulatory RNAs (e.g., miRNAs) sequestrating miRNA from their endogenous targets; and (4) scaffold lncRNAs when they serve as adaptors to enhance protein-RNA, protein-DNA, or protein-protein interactions [7, 12, 13, 23]. Taken together, experimental data suggest that lncRNAs have key roles in all aspects of gene regulation through diverse mechanisms of action, depending on their subcellular localization as well as their interactions with their protein partners, other RNA molecules, or DNA molecules. A summary of divergent mechanisms of action of lncRNA in diabetic nephropathy (DN) is listed in Table 2.

An important recent advance in understating the mechanism of action of lncRNAs is that contrary to what was previously believed to be a sine qua non definition of lncRNAs, some lncRNAs have small open reading frame transcribed into functional micropeptide [24–27]. An example is LINC-00984 which has been recently shown to encode myoregulin (MLN), a very short micropeptide, which turned out to be functional and implicated in inhibition of the sarco-/endoplasmic reticulum calcium transport ATPase calcium-ATPase to regulate calcium handling in muscle cells [28–31]. Furthermore, a skeleton- and heart muscle-specific lncRNA known as LINC00116 was shown to encode a short micropeptide, mitoregulin (Mtln), with a crucial role in mitochondrial function [32–34].

Identification and Characterization of lncRNAs

Compared to mRNAs, lncRNAs are typically expressed at lower levels, with more tissue specificity and less evolution conservation [10, 11], making it challenging to be easily examined, but recent technological developments, especially advances in next-generation sequencing, have provided multiple approaches to identify and annotate lncRNAs.

A traditional method of identifying lncRNAs has been to take advantage of cDNA libraries, where lists of RNA transcripts are created after generation and sequencing of cDNA libraries. An alternative method to identify lncRNAs has been to use serial analysis of gene expression, a high-throughput sequencing method, which is based on generating short stretches of cDNA sequences (serial analysis of gene expression tags) containing restriction enzyme sites at the 3′ end of transcripts. Cap analysis of gene expression is also an alternative, high-throughput sequencing method, where 5′-capped RNAs can be quantified for expression and identified simultaneously [7, 9, 35]. More recently, RNA-seq has become a powerful strategy for RNA expression profiling and identification of novel lncRNAs. A number of single-cell transcript sequencing methods have more recently been adapted to detect heterogenic gene expression and for small sample sizes. In the meantime, other specialized methods were designed to map RNA transcripts undergoing degradation (such as lncRNAs serving as host genes for miRNA where miRNAs are generated upon lncRNA degradation), map all transcript isoforms, or measure the half-life

of transcripts, and assess the lncRNA decay rates in vivo [9, 35].

Importantly, a number of online databases have been created to study the structure and function of different lncRNAs. These databases include lncRNAdb (http://www.lncRNAdb.org/) and LNCipedia (http://www.lncipedia.org) that provides information on the sequence and structure of human lncRNA. ChIPBase (http://rna.sysu.edu.cn/chipbase/) database helps study transcription factor binding sites, and NRED (http://nred.matticklab.com/cgi-bin/ncrnadb.pl) helps with the expression of human and mouse lncRNAs.

To further characterize the biological functions of an lncRNA transcript, gain-of-function or loss-of-function strategy is commonly employed [7, 9]. More recently, some state-of-the-art techniques, such as CRISPR and proximity-labeling, have been incorporated into functional characterization of lncRNAs. Liu et al. [36] have developed a genome-wide CRISPR-mediated interference screening platform, where a library of 16,401 lncRNA genes each targeted by 10 single guide RNAs, were applied to screen their function on cell growth in 7 human cell lines. Similarly and by using proximity labeling of RNA with the peroxidase enzyme APEX2 followed by RNA-seq), Fazal et al. [37] generated a nanometer resolution spatial map of the human transcriptome in 9 distinct subcellular localization, where a number of lncRNAs were attributed to a variety of novel functions.

lncRNAs and the Pathogenesis of Kidney Diseases

CKD is a major public health problem worldwide with exponentially increasing prevalence. Therefore, it is crucial to identify novel therapeutic targets associated with the development and progression of CKD.

An emerging body of evidence indicates that lncRNAs could play key regulatory roles in diverse kidney pathologies, including in CKD progression and development, acute kidney injury (AKI), and renal cell carcinoma (RCC), suggesting a high potential for lncRNAs as promising novel targets in future treatments and diagnostic approaches in kidney diseases. In support of a central role of lncRNA in kidney diseases, NEAT1, an lncRNA important for the innate immune response, was initially reported to be correlating with the severity of AKI in sepsis [38]. The mechanism involved in its pathogenic effect was proposed to be based on its role in inflammation and apoptosis since NEAT1 plays a crucial role in modulating p50 and p65 (NF-κB activity markers). Notably, the positive regulation of NEAT1 increases reactive oxygen species and pro-inflammatory cytokines. NEAT1 also upregulates Bax and caspases 3 and 9, resulting in enhanced apoptosis [38]. Another important lncRNA in the pathogenesis of AKI is PRINS, an lncRNA induced by HIF-1α following hypoxia [39]. PRINS interacts with regulated on activation, normal T-cell expressed and secreted, causing inflammation that promotes ischemic reperfusion injury development. Additionally, PRINS is a well-established apoptosis regulator that further increases cell death in ischemic reperfusion injury [39].

Glomerulosclerosis and renal fibrogenesis are key conditions associated with progression of CKD and ESRD. Recent evidence suggests that lncRNA lnc-TSI downregulates a pivotal pathway to fibrosis [40]. lnc-TSI binds to Smad3, inhibiting the phosphorylation of TGF-β1. To further prove the impact on TGF-β1, human Lnc-TSI was delivered into a progressive kidney fibrosis model of unilateral ureteral obstruction, resulting in improving renal fibrosis. Furthermore, lnc-TSI could also serve as a biomarker since it was concluded that patients with IgA nephropathy who started with lower expression levels of lnc-TSI in their kidney biopsies exhibited a more rapid progression of renal fibrosis [40].

Another important lncRNA, HOTAIR, has been shown to play an essential role in RCC pathogenesis through chromatin remodeling [41]. It was demonstrated that knockdown of HOTAIR by small interfering RNA, significantly lowered proliferation and invasion of RCC in vivo and in vitro, by decreasing the expression of EZH2 and recruitment of H3K27me3. Furthermore, important indicators of cell growth proliferation (p53, p21, and p16) were dysregulated in the HOTAIR knockout animal, possibly due to loss of recruitment of chromatin-remodeling complex by HOTAIR, leading to derepression of p53, p21, and p16 expression by H3K27me3 and EZH2 [41].

FoxO3-induced lncRNA 1 (FILNC1) also plays a critical role in the development of RCC through inhibition of c-Myc-mediated energy metabolism [42]. FILCN1 expression is downregulated in RCC in vitro and in vivo, leading to the inhibition of glucose-induced apoptosis and the promotion of proliferation under low-glucose conditions. Mechanistically, FILCN1 acts as a decoy to block AU-rich element RNA-binding protein 1 (AUF1) from binding to c-Myc mRNA, resulting in reduced glucose consumption. FILCN1-deficient mice showed greater tumor size and weight than control. Importantly, the relation between FILCN1 concentration and the clinical outcome was directly proportional in RCC patients, further demonstrating the crucial role of FILCN1 in RCC

[42]. Finally, Wu and colleagues [43] have recently explored lncRNA expression profiling in RCC with 141 patients (71 with ccRCC and 62 controls). A 5-lncRNA signature, including lncRNA-LET, PVT1, PANDAR, PTENP1, and linc00963, were identified and validated in the training set and testing set, respectively, from patients' serums [43], showing the great potential that lncRNA has for clinical use.

lncRNA in Diabetic Nephropathy

Diabetic nephropathy (DN) is the leading cause of CKD and ESRD in the world [44, 45]. A growing number of published studies suggest that lncRNAs play key roles in the development and progression of DN [46–49]. Interestingly, many lncRNAs were linked to mitochondrial function, highlighting the importance of mitochondrial dysfunction in progression of DN [46, 49, 50].

Among dysregulated lncRNAs implicated in the development of DN, lncRNA Erbb4-IR is reported to have a major role in the pathogenesis of DN though a TGF-β/Smad3-dependent pathway [51]. Erbb4-IR is a Smad-3-dependent lncRNA that is upregulated by advanced glycosylation end products. The knockdown of Erbb4-IR protected db/db diabetic mice, an established murine model of type 2 diabetes (T2D), against proteinuria and kidney fibrosis. Indeed, collagen I and IV production in tubular and mesangial cells was markedly decreased in Erbb4-IR knockdown diabetic mice compared to the controls [51]. Mechanistically, Erbb4-IR seems to act as a decoy for the suppression of miR-29b, an antifibrotic miRNA [52], exacerbating renal fibrosis in DN. These results open the possibility of targeting Erbb4-IR to mitigate DN progression [53].

Another key lncRNA in DN progression is maternally expressed gene 3 (MEG3) [54]. Functional studies have shown that MEG3 has antiproliferative function, as shown in a variety of cancers [55], but MEG3 is also involved in insulin resistance and accelerated senescence in type 2 diabetic patients [56]. Mechanistically, MEG3 is upregulated in diabetes, and it acts as a decoy against miR-145 [54]. Importantly, knockdown of MEG3 decreased expression levels of collagen IV and fibronectin in serum and kidney tissues, leading to attenuated mesangial fibrosis and significant improvement in key features of DN. MEG3 could also serve as a sponge for miR-181a, promoting inflammation and fibrosis through an Egr-1/TLR4 signaling [57]. These results suggest that MEG3 has a pathogenic effect on DN by promoting inflammation and fibrosis.

GAS5 is another lncRNA involved in DN progression [56]. GAS5 seems to act as an miRNA sponge to protect the kidney against mesangial proliferation and fibrosis [58]. GAS5 concentration was reported to be lower in DN patients [58]. This novel lncRNA has 2 major pathways to mitigate mesangial cell proliferation and fibrosis markers, including fibronectin, collagen IV, and TGF-β1 expression. On the one hand, GAS5 sponges miR-221, an miRNA responsible for upregulating fibrotic molecules in mesangial cells by directly binding to sirtuin-1 (SIRT1), a renoprotective molecule in DN [59]. On the other hand, GAS5 directly upregulates SIRT1 expression. Both pathways upregulate the capacity of sirtuin-1 to reduce tubular, podocyte, and mesangial damage in DN [58]. GAS5 was also recently shown to downregulate matrix metalloproteinase 9 (MMP9), by recruiting EZH2 to the promoter region. GAS5 downregulation of MMP9 was able to reduce fibrotic markers (TGF-β1 and collagens I and III). Furthermore, the biochemical indicators of progression of DN, such as serum creatinine, BUN, and 24-h proteinuria were significantly improved, confirming the utility of GAS5 as an anti-fibrotic molecule that could improve the DN phenotype [60].

XIST, an lncRNA first reported to participate in X-chromosome inactivation, was also reported to be involved in DN progression [61]. XIST expression in the diabetic kidney is high, and its knockdown protects the kidney against interstitial fibrosis [61]. Mechanistically, XIST acts as decoy for miR-93-5p, an miRNA previously shown to be involved in the progression of DN by targeting VEGFA [62] and Msk2 [63]. XIST could downregulate the expression of miR-93-5p, which also targets CDKN1A [61]. Therefore, in DN, high expression levels of XIST allow CDKN1A to induce renal damage and fibrosis by blocking miR-93-5p inhibition [61].

Kato and colleagues [64] recently identified the host lncRNA of a megacluster of nearly 40 miRNAs could be central to the development of DN. The authors convincingly show that lncRNA lncMGC expression is upregulated under high glucose or TGF-β1 stimulation. Indeed, the authors show that lncMGC controls the expression of the miR-379 cluster, and the upregulation of this miRNAs cluster induces ECM accumulation and hypertrophy in DN [64].

PGC-1α (a coactivator of PPARγ, peroxisome proliferator-activated receptor gamma) is reported to be downregulated in DN, and a key factor implicated in mitochondrial dysfunction in DN progression [65–67]. We have recently shown that taurine upregulated gene 1 (Tug1), an lncRNA found in chromosome 22q12 [68],

regulates the expression of PGC-1α [46]. Tug-1 exhibits a renoprotective phenotype in DN by upregulating PGC-1α and improving mitochondrial bioenergetics and mitochondrial reactive oxygen species production under high-glucose conditions. Transgenic expression of Tug1 in diabetic mice (db/db) mice improved GBM thickening and reduced podocyte apoptosis, leading to amelioration of albuminuria and DN progression [46]. In human patients, lower levels of TUG-1 expression were correlated with reduced levels of estimated glomerular filtration rate [46, 50]. Tug-1 expression aberration also contributes to podocyte apoptosis [69], possibly by inhibiting the transcription factor CHOP (C/EBP homologous protein), a known regulator of ER stress and apoptosis. Deficiency of Tug-1 leads to PGC-1α downregulation and a significant increase in apoptosis [69]. TUG1 can also sponge miR27a-3p, avoiding its pro-inflammatory, profibrotic, and proapoptotic activities [70].

Another lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) expression was shown to correlate with podocyte apoptosis under high-glucose conditions [71]. MALAT1 is involved in a feedback loop between β-catenin and serine/arginine splicing factor 1 (SRSF1), a MALAT1 RNA-binding protein 1, which leads to podocyte impairment. MALAT1 is able to disrupt the cycle, leading to improvement in podocyte apoptosis [71]. In another key observation, Li et al. [72] also provided strong evidence indicating that MALAT1 expression is substantially increased in isolated tubular cells from STZ rats and in cultured HK-2 tubular cells treated with high glucose. Their findings suggest that elevated MALAT1 expression, acting as a decoy for miR-23c, suppresses miR-23c, leading to the derepression of miR-23c target ELAVL1, which in turn targets the pro-inflammation gene NLRP3 and ultimately leads to a pro-inflammatory programmed cell death.

Finally, nuclear enriched abundant transcript-1 (NEAT1) was reported to accelerate renal fibrosis in DN by directly activating the Akt/mTOR signaling pathway [73–75]. Knockdown of NEAT1 decreased ECM protein secretion in mesangial cells and repressed mRNA expression of TGF-β1, fibronectin, and collagen IV even under high-glucose conditions [74]. NEAT1 can also act as a decoy-promoting extracellular matrix accumulation and epithelial-mesenchymal transition by targeting miR-27b-3p and ZEB1 [76]. These studies also provided evidence indicating that NEAT1 knockdown reduced renal damage in DN mice, suggesting a critical role of this lncRNA in fibrogenesis, cell proliferation, and renal impairment in DN.

lncRNAs and Their Promise as Biomarkers and/or Therapeutic Targets in Kidney Diseases

Because of their tissue-/cell-specific expression, as well as their developmental state-specific pattern, lncRNAs could potentially serve as exceptional biomarkers for multiple pathologies. Indeed, some lncRNAs have already been established as reliable diagnostic biomarkers. A recent example is the lncRNA prostate cancer antigen 3 (PCA3), a prostate-specific lncRNA, overexpressed in ~95% of prostate cancer cases and currently being used as a marker in prostate cancer [77]. Similarly, long intergenic ncRNA predicting cardiac remodeling (LIPCAR), a mitochondrial lncRNA, has been shown to predict cardiovascular death after MI [78]. Importantly and in regard to association of lncRNAs with CKDs, the urinary TUG1 level was recently found to be significantly lower in patients with focal segmental glomerulosclerosis. Indeed, it was suggested that the loss of TUG1 expression in urinary sediment could strongly be associated with focal segmental glomerulosclerosis progression, indicating that TUG1 could be a noninvasive biomarker for assessing podocytopathies [79].

A growing body of evidence is also examining the role of lncRNAs in the development of diabetes and DN progression. For instance, a recent study examined lncRNAs present in the peripheral blood samples of type 2 diabetic patients and normal controls and identified 17 differentially expressed lncRNAs between the 2 groups [80]. Further characterization and human studies discovered that lncRNA ENST00000550337.1 could serve as a reliable marker for prediabetes and the development of T2D. Another study has recently shown that lncRNA-ARAP1-AS2 is elevated, while lncRNA-ARAP-AS2 is decreased in diabetic patients with DN, suggesting that they may also serve as biomarkers for the development of DN [81].

However, despite the growing potential use of lncRNAs as clinical biomarkers, much less is known about the role of lncRNAs as therapeutic targets. In the last decade, we have seen major advances in targeting RNA in vivo, which has changed the landscape of drug development with several pharmaceutical companies, including Alnylam Pharmaceuticals (https://www.alnylam.com/), Avidity Biosciences (http://www.aviditybiosciences.com), The Medicines Company (now part of Novartis), miRagen (http://www.miragen.com/), and Regulus (http://regulusrx.com/) at the forefront of a set of new technologies targeting RNAs in patients with a wide variety of pathologies. These companies and other investiga-

tors use a variety of techniques to target ncRNAs. While some of these companies focus on modulating miRNAs using oligonucleotides, others target lncRNAs by using double-stranded RNA-mediated interferences (RNAi), single-stranded antisense oligonucleotide (ASO)-based strategies, or CRISPR/Cas9 gene-editing systems. Each of these strategies has its own benefits and pitfalls. For example, the RNAi technology initiates degradation of the RNA that utilizes the multiprotein RNA-induced silencing complex containing Dicer, TRBP, and Ago2 proteins. Despite great success in knocking down a number of lncRNAs using the RNAi technology in cell lines, silencing lncRNAs in *in* vivo experiments has turned out to be more challenging [82]. This is in part due to the lack of efficient delivery methods in vivo. Further preclinical clinical studies are underway to assess the value and limitations of silencing lncRNAs using RNAi strategies in vivo [83]. As an example of using RNAi technology in vivo, it was recently shown that the knockdown of lncRNA H19 LNA-GapmeRs in vivo significantly limited aneurysm growth in 2 murine abdominal aortic aneurysm models. Correlation of upregulated H19 with smooth muscle cell content and SMC apoptosis was observed in human abdominal aortic aneurysm tissue samples and in a preclinical $LDLR^{-/-}$ Yucatan mini-pig aneurysm model [84].

An ASO-based approach exhibits a different mode of action where classically ASOs bind to their target RNAs mainly based on their complementary sequence, leading to inhibition or alteration of their gene expression via steric hindrance, splicing alterations, and initiation of target degradation [82]. Similar to the use of RNAi, however, despite great promise of ASOs and its modified versions, several key limitations of ASO's delivery and off-target issues remain to be carefully addressed. Finally, the use of CRISPR/Cas9, nuclease guidance technology, has already revolutionized a variety of gene-editing and gene-targeting applications. As its name indicates, the system has 2 components: a bacterial endonuclease enzyme (typically Cas9) and a guide RNA that directs Cas9 to a desired site in the genome, leading to targeted double-stranded DNA breaks at a specific site.

The CRISPR/Cas9 technology can also be used to drive overexpression and transcriptional repression of lncRNA. This novel genome-editing technology has been used to drive high-throughput screening of lncRNAs involved in many diseases, particularly in cancer studies [85]. CRISPR/Cas9 editing of protein coding gene is generally more straightforward because deletion/insertion generated by this genome editing technology usually causes frameshift or premature stop of the protein coding gene; however, such deletion/insertion is not likely to alter the function of the lncRNA. Therefore, it is necessary to either delete the entire lncRNA genome or, as some researchers had recently demonstrated, target the lncRNA splice acceptor/donor sites [86–88]. Overall, several key challenges remain to be addressed, despite the potential use of CRISPR as a novel gene-editing system in gene therapy, including how to properly deliver CRISPR to specific tissues and its off-target effects [89, 90].

Conclusions

Although extensive research has elucidated the functional importance of a number of lncRNAs, there is a vast area of opportunity to further extend the understanding of this novel class of RNA molecules. We believe that the potential applications of lncRNA in kidney therapeutics are virtually limitless, and the human benefits of researching these molecules are already presented with promising biomarkers and therapeutic targets that could soon improve outcomes in a wide variety of renal diseases, including DN.

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Conflict of Interest Statement

The authors have no conflicts of interests.

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Author Contributions

J.D.C., J.L., and F.R.D. all contributed to writing of this manuscript. All authors have read and approved the final manuscript.

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