Values and Limitations of Targeting lncRNAs in Diabetic Nephropathy

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COMMENTARY

Deep RNA sequencing of mammalian transcriptomes during the past two decades has unveiled more than 100,000 different RNAs, far more than the estimated 20,000 protein-coding genes, suggesting that the protein-coding potential of the mammalian genome is very limited and the majority of these RNA sequences are indeed noncoding (1–3). Although once considered "evolutionary junk" or "transcriptional noise," more recent observations have shown that noncoding RNAs (ncRNAs) participate in diverse biological activities of the cell by interacting with RNA, DNA, or proteins to modulate the expression of protein-coding genes. Generally, ncRNAs are divided into two major subclasses (long or short), depending on their size. The long ncRNAs (lncRNAs) are arbitrarily defined as transcripts longer than 200 nucleotides that are produced by RNA polymerase II and lack protein-coding potential, thus separating them from the other subclass of small ncRNAs that range from a few to 200 nucleotides and include microRNAs (miRNAs), small nuclear RNAs, circular RNAs, and small nucleolar RNAs (4). Compared with messenger RNAs (mRNAs), lncRNAs are usually less evolutionarily conserved, less abundant, and more tissue specific (5). Cumulative research has revealed that lncRNAs exert a wide variety of biological functions and their aberrant expression has been associated with diverse pathologies including cancer and cardiac, neurological, and metabolic diseases (6–8). Mechanisms underlying the broad functions of lncRNAs are still emerging. It has recently become apparent that lncRNAs function in some cases as transcriptional regulators (4,9,10), whereas other lncRNAs appear to function as scaffolds or act as decoys to inhibit proteins or miRNAs (4,9,10). Despite all these advances, the molecular functions of lncRNAs in many human diseases remain elusive, and more detailed functional studies are needed to unravel the biological roles of lncRNAs.

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease in the U.S. (11). As current treatment options for DN remain limited, new and effective therapeutic approaches are needed, including those that target lncRNAs (12). To this end, Kato et al. (13) have recently identified an lncRNA, lnc-MGC, that serves as a scaffold for a cluster of 40 miRNAs and appears to induce features of early type 1 DN. In addition, we have recently identified the role of another lncRNA, Tug1 (taurine upregulated gene 1), in DN by performing unbiased RNA-Seq profiling of kidney glomeruli using the *db/db* mouse model of type 2 DN (T2DN). We found that TUG1 levels are downregulated in human DN and that podocyte-specific transgenic expression of Tug1 in diabetic mice prevented biochemical and histological features of DN (14).

In this issue of *Diabetes*, Sun et al. (15) describe the characterization of a novel pathogenic lncRNA, *Erbb4-IR*, in the *db/db* mouse model of T2DN. *Erbb4-IR* promotes transforming growth factor-β (TGF-β)/Smad signaling, one of the most critical signaling pathways promoting kidney fibrosis in DN (16,17). Taking advantage of high-throughput RNA-Seq in Smad3 knockout (KO) mice, the authors had previously identified 21 differentially expressed lncRNAs related to renal inflammation and fibrosis in mouse models of unilateral ureteral obstructive nephropathy and antiglomerular basement membrane glomerulonephritis (18). lncRNA *Erbb4-IR* (np_5318), located within the intron region between the first and second exon of mouse *Erbb4* gene on chromosome 1, was one of the common Smad3-dependent lncRNAs whose expression was significantly upregulated in wild-type mice but repressed in Smad3 KO mice in both models of kidney injury (18). Interestingly, chromatin immunoprecipitation demonstrated that the Smad3 protein directly binds to the conserved promoter region of *Erbb4-IR*, indicating that *Erbb4-IR* is a direct target gene of Smad3 (18). To explore the possible functional roles of this novel lncRNA in T2DN, the authors found that *Erbb4-IR* levels were significantly upregulated in the kidneys of wild-type mice but not in Smad3 KO *db/db* mice (15). In situ hybridization and fluorescence in situ hybridization assay suggested that *Erbb4-IR* is mainly

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expressed in the nucleus of mesangial and tubular epithelial cells. Expression of *Erbb4-IR* was specifically induced by advanced glycation end products via a Smad3-dependent mechanism but not by high glucose. Importantly, kidney-specific silencing of *Erbb4-IR* largely improved kidney histology and decreased albuminuria in *db/db* mice, presumably due to suppression of TGF-β/Smad3–mediated renal fibrosis (15). Mechanistically, the authors provided evidence that lncRNA *Erbb4-IR* functions as a decoy to inhibit *miR-29b* (15), an established renoprotective miRNA involved in the progression of TGF-β/Smad–dependent renal fibrosis under diabetic and nondiabetic conditions (19,20).

Despite these novel observations, Sun et al. (15) leave some interesting questions unanswered for future studies. For instance, there seems to be a feed-forward loop between TGF-β/Smad3 signaling and lncRNA *Erbb4-IR*, as *Erbb4-IR* expression was positively regulated by advanced glycation end products via TGF-β/Smad3 signaling, whereas knockdown of *Erbb4-IR* inhibited TGF-β1 mRNA expression and Smad3 activation in *db/db* mice (15). It would be interesting to explore whether gain of function of *Erbb4-IR* could promote TGF-β/Smad–mediated renal fibrosis in vitro and in vivo. Furthermore, as the putative binding site of *Erbb4-IR* is very short in the *miR-29b* gene (about 50 nucleotides) (15), it would also be important to examine the structure–function relationship between *Erbb4-IR* and *miR-29b* and to explore whether the long region (about 2kb) of *Erbb4-IR* could bind to *miR-29b* via other sites or bind to other miRNAs to contribute to the repression of *miR-29b*.

Because of the previously published work (13,14), as well as the work described by Sun et al. (15), there is increasing recognition that lncRNAs could be potential novel therapeutic targets for DN and other kidney diseases. Loss of function for the pathogenic lncRNAs, such as *lnc-MGC* and *Erbb4-IR*, or gain of function for protective lncRNAs, such as *Tug1*, could be novel pharmacological intervention strategies to improve key biochemical and histological features in micro- and macrovascular complications of diabetes. However, because similarity between mouse and human lncRNAs sequences is modest, it would also be critical to understand the higher order structure of specific lncRNAs (21).

In summary, Sun et al. (15) identified *Erbb4-IR* as a Smad3-dependent profibrotic lncRNA, which promotes the progression of renal fibrosis in T2DN by serving as a decoy to suppress the expression of antifibrotic *miR-29b*. These results provide additional insights into the values and/or limitations of targeting lncRNAs in DN (13,14). It is intriguing to speculate that we could eventually target specific tissues pharmacologically by targeting tissue-specific lncRNAs and use lncRNAs as novel and specific therapeutic targets for DN and other kidney diseases.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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