

The long noncoding RNA *Tug1* connects metabolic changes with kidney disease in podocytes

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An increasing amount of evidence suggests that metabolic alterations play a key role in chronic kidney disease (CKD) pathogenesis. In this issue of the *JCI*, Long et al. report that the long noncoding RNA (lncRNA) taurine-upregulated 1 (*Tug1*) contributes to CKD development. The authors show that *Tug1* regulates mitochondrial function in podocytes by epigenetic targeting of expression of the transcription factor PPAR γ coactivator 1 α (PGC-1 α , encoded by *Ppargc1a*). Transgenic overexpression of *Tug1* specifically in podocytes ameliorated diabetes-induced CKD in mice. Together, these results highlight an important connection between lncRNA-mediated metabolic alterations in podocytes and kidney disease development.

lncRNAs: not just noise

While less than 2% of the genome is translated into proteins, recent advances in RNA-sequencing (RNA-seq) technologies have revealed that approximately three-quarters of the mammalian genome is transcribed into RNA. The majority of these transcripts have no protein-coding potential (1); however, several roles and families of these nonprotein-coding RNA species have been deciphered. Long noncoding RNAs (lncRNAs) encompass a large and diverse class of transcribed RNA molecules that are longer than 200 nucleotides and do not encode proteins. Initially, lncRNAs were considered transcriptional noise. However, evidence is accumulating that the majority of noncoding RNAs are functional (2). Whole-genome sequencing has detected approximately 30,000 lncRNA transcripts to date, and studies have revealed that these lncRNAs are species specific, overall poorly conserved, and expressed at low levels. Unfortunately, fewer than 300 lncRNAs have been

functionally annotated in the Long Noncoding RNA Database (3). lncRNAs have several modes of action but are generally considered important transcriptional regulators. Enhancer RNAs are considered a subtype of lncRNA that are transcribed from the enhancer region and physically involved in looping the enhancer and promoter regions to regulate transcription. Some lncRNAs can act through chromatin remodeling (mostly by altering the polycomb-repressive complex 2 [PRC2]) to achieve transcriptional regulation. Other lncRNAs interfere with splicing, RNA stability, or translation (Figure 1).

The Cancer Genome Project has highlighted differential expression of lncRNAs in multiple cancer types, and lncRNA signatures are emerging as novel diagnostic cancer biomarkers, as most cancer types are characterized by dysregulated expression of several hundred specific lncRNA species. Conversely, little is known about lncRNA functions and regulations in non-cancerous tissues. In this issue, Long et

al. (4) performed RNA-seq on glomeruli from diabetic mice, specifically focusing on identifying changes in lncRNA levels compared with those in nondiabetic animals, and found that taurine-upregulated 1 (*Tug1*) levels were consistently reduced in diabetic mouse glomeruli. While most lncRNAs show species-specific expression and regulation, *Tug1* expression was found to be evolutionally conserved and was also decreased in human diabetic kidney samples (4). Long et al. localized the *Tug1*-specific changes to the podocytes and therefore set out to further study the role of *Tug1* in podocytes and in development. Using animal and cell culture models, Long and colleagues demonstrated that *Tug1* regulates expression in podocytes of the transcription coactivator PPAR γ coactivator 1 α (PGC-1 α), which regulates genes involved in energy metabolism. According to their data, *Tug1* acts as a bridge between the 400-kb upstream enhancer and promoter of *Pgc1a*, thereby regulating PGC-1 α expression (4). In a high-glucose environment, *Tug1* expression was decreased, resulting in decreased *Pgc1a* transcription in podocytes.

Podocytes in diabetes

Podocytes are specialized glomerular epithelial cells that form a layer of the filtration barrier in the kidney. Podocytes are terminally differentiated with limited capacity to renew. In animal models, podocyte injury and loss occurs early during the course of diabetes (5). Low podocyte numbers are a strong predictor of future functional decline in human subjects with either type 1 or type 2 diabetes; therefore, the identification of early metabolic changes in podocytes is thought to be critical for understanding the pathogenesis of diabetic kidney disease (6).

Recent studies indicate that podocytes are insulin sensitive and utilize glucose as their energy source. Podocyte-specific insulin receptor-KO mice develop severe

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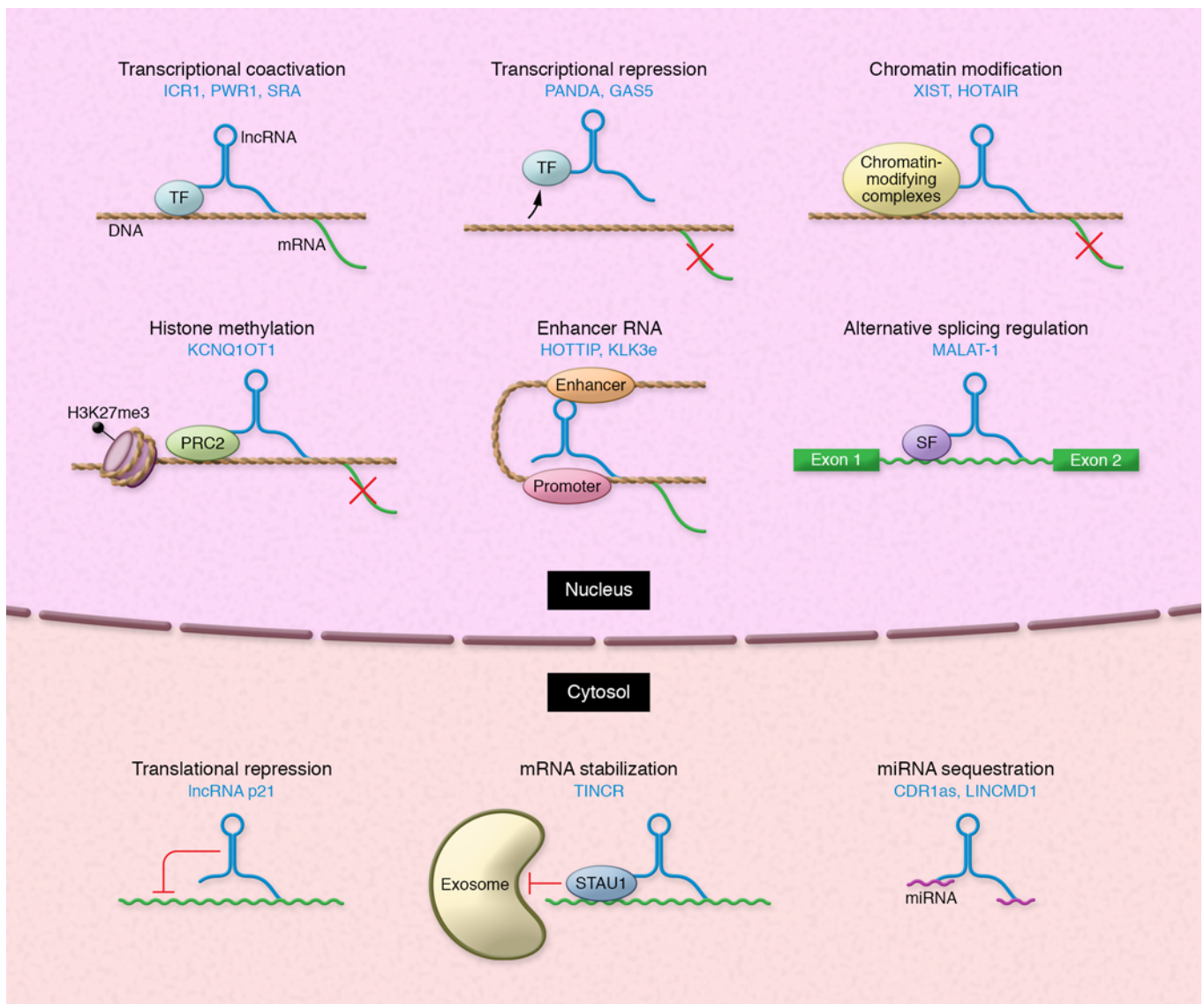


Figure 1. IncRNAs can interfere with gene function at multiple levels. Studies have described a range of mechanisms by which lncRNAs regulate their targets. In the cell nucleus, lncRNAs can regulate transcription by recruiting chromatin-modifying complexes and transcriptional factors to silence or enhance target gene expression. Additionally, some lncRNAs regulate gene expression by influencing pre-mRNA splicing. In the cytosol, lncRNAs can regulate mRNA expression by altering mRNA stability, mRNA translation, or by competing for microRNA binding. ICR1, Interfering Crick RNA 1; PWR1, Promoting Watson RNA1; SRA, Steroid receptor RNA Activator; PANDA, P21 associated ncRNA DNA damage activated, PANDA antisense RNA; GAS5, Growth arrest-specific 5; TF, Transcription factor; XIST, X-inactive specific transcript; HOTAIR, HOX transcript antisense RNA; KCNQ10T1, KCNQ1 overlapping transcript 1; PRC2, Polycomb Repressive Complex 2; HOTTIP, HOXA transcript at the distal tip; KLK3e, Kallikrein-related peptidase 3 eRNA; SF, Splicing factor; STAU1, Staufen Double-Stranded RNA Binding Protein 1; TINCR, Tissue Differentiation-Inducing Non-Protein Coding RNA; CDR1as, Cerebellar degeneration-related protein 1 Antisense RNA; LINCMD1, long intergenic non-protein coding RNA, muscle differentiation 1.

glomerulosclerosis, even in the absence of hyperglycemia (7). The effect of insulin receptor deletion appears to be independent of glucose uptake, as mice with podocyte-specific deletion of glucose transporter type 4 (*Glut4*) do not develop similar changes (8). Insulin-induced activation of AKT acts as an important regulator of cytoskeletal changes and pro-survival factor in podocytes and might explain the strong phenotype develop-

ment in the podocyte-specific insulin receptor-KO mice (9). Insulin also regulates mitochondrial function via the transcription factor FOXO (10).

In addition to glucose utilization, proper mitochondrial function is essential for podocyte health. Patients with mutations in genes encoding different mitochondrial enzymes, such as COQ2, COQ6, COQ10, and aarF domain-containing kinase 4 (ADCK4), present with podocy-

topathy and chronic kidney disease (CKD) (11). In animal models, genetic deletion of mitochondrial transcription factors such as Kruppel-like factor 6 (KLF6) specifically from podocytes not only causes mitochondrial defects but is sufficient to induce glomerulosclerosis and kidney failure (12).

In diabetic animal models, both quantitative and qualitative mitochondrial defects have been described and thought to contribute to CKD develop-

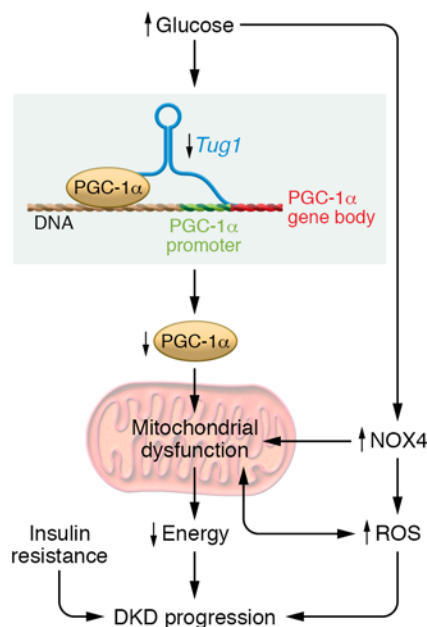


Figure 2. Proper mitochondrial function is essential for podocyte health in diabetes. *Tug1* expression is decreased in diabetic podocytes. *Tug1* regulates PGC-1 α expression by epigenetic targeting. Decreased PGC-1 α levels results in structural and functional mitochondrial changes. Impaired mitochondrial function will cause energy depletion, increased ROS generation, and, ultimately, the development of diabetic kidney disease. DKD, diabetic kidney disease; NOX4, NADPH oxidase 4.

ment. Healthy mitochondria are believed to be elongated and filamentous. In contrast, diabetic podocytes are characterized by small, fragmented mitochondria (13), indicating a potential fission problem. The kinase Rho-associated coiled coil-containing protein kinase 1 (ROCK1) has emerged as an important mediator of mitochondrial fission in diabetes mellitus (13). High glucose levels activate ROCK1, which then recruits the GTPase dynamin-related protein 1 (DRP1) from the cytoplasm to the mitochondrial outer membrane, thereby inducing mitochondrial fragmentation (13). In diabetic mice, *Rock1* deletion can restore mitochondrial morphology and function as well as attenuate albuminuria.

Podocytes isolated from diabetic mouse models show alterations in mitochondrial gene expression (14), including genes encoding upstream transcription factors, such as PGC-1 α and transcription factor A, mitochondrial (TFAM), that are key regulators of mitochondrial biogene-

sis and gene expression. PGC-1 α expression is lower in diabetic glomeruli, and the level of PGC-1 α correlates with structural and functional damage. The expression and regulation of PGC-1 α is complex and includes transcriptional and post-transcriptional changes. Epigenetic regulation of PGC-1 α has also been described, including cytosine methylation changes in addition to the *Tug1*-induced changes reported by Long et al.

Tug1 does it in vivo

Like most other lncRNAs, the functional role of TUG1 has been poorly understood. TUG1 expression is increased in most cancer types, and knockdown of TUG1 in cultured cancer cells has been shown to suppress cell proliferation, invasion, and colony formation (15). Given that *Tug1* has multiple targets and can interfere with several steps in the process of carcinogenesis, it has been proposed as a therapeutic target. Despite overall poor conservation between species, several lncRNAs are ultraconserved. Metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) is conserved between human and mice. *MALAT1* expression is increased in human cancer samples. *MALAT1*-deficient mice have no phenotype at baseline; however, these animals are resistant to breast and lung cancer development. Furthermore, *MALAT1*-targeting antisense oligonucleotides have shown efficacy in a preclinical breast cancer model, indicating the therapeutic potential for targeting this lncRNA (16). Like *MALAT1*, *Tug1* appears to be evolutionarily conserved between mice and humans and similarly regulated in both species.

To explore the function of *Tug1* in podocytes and diabetic kidney disease, Long et al. generated transgenic mice that overexpress *Tug1* specifically in podocytes (4). These animals appeared healthy at baseline and were crossed with *db/db* mice, which serve as a model of diabetic nephropathy. Podocyte-specific overexpression of *Tug1* attenuated diabetes-induced ROS formation, albuminuria, and histopathological changes. Long and colleagues propose that the beneficial effect of *Tug1* is fully mediated by restoration of PGC-1 α expression, which restores mitochondrial bioenergetics in podocytes. Together, these results highlight a previously unknown role for *Tug1*.

The bigger picture

The study by Long et al. (4) is fully consistent with recent studies indicating that mitochondrial health and PGC-1 α play a critical role in the kidney. For example, gene expression studies have identified mitochondrial transcripts, including those associated with fatty acid oxidation, as one of the most differentially expressed gene groups in CKD (17). Transgenic expression of *Pgc1a* in renal tubule cells protects mice from both acute and chronic kidney injury (17, 18). The work by Long et al. now shows that the PGC-1 α mitochondrial axis is equally important in podocytes (Figure 2) and provides novel mechanistic insight into PGC-1 α regulation. By identifying a lncRNA with podocyte-specific function, this study is an important first step in understanding how these noncoding RNAs regulate kidney function. Future studies aimed at understanding the role of lncRNAs and metabolic changes in CKD have the potential to provide important insight and possible therapeutic targets for treating kidney disease.

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