The potential role of IncRNAs in diabetes and diabetic microvascular complications

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Abstract. Long noncoding RNAs (lncRNAs) are a group of noncoding RNAs that are longer than 200 nucleotides without protein-coding potential. Because of which these RNAs have no significant protein-coding potential, they were initially considered as "junk-products" of transcription without biological meaning. Nevertheless, recent research advancements have shown that lncRNAs are involved in many physiological processes such as cell cycle regulation, cell apoptosis and survival, cancer migration and metabolism. This review described the function of IncRNAs and the potential underlying mechanism involved in diabetes and diabetic microvascular complications. The roles of lncRNAs in the pathogenesis of type 2 diabetes mellitus have only recently been recognized, involving hepatic glucose production and insulin resistance. We further investigated the mechanisms of lncRNAs in diabetic nephropathy (DN), including the roles of lncRNAs in mesangial cells (MCs) proliferation and fibrosis, inflammatory processes, extracellular matrix accumulation in the glomeruli and tubular injury. We also discussed the potential mechanism of lncRNAs in diabetic retinopathy (DR), including aberrant neovascularization and neuronal dysfunction. This review summarized the current knowledge of the functions and underlying mechanisms of lncRNAs in type 2 diabetes mellitus and related renal and retinal complications. Accumulating evidence suggests the potential of lncRNAs as therapeutic targets for clinical applications in the management of diabetes.

Key words: LncRNAs, Diabetes, Diabetic microvascular complications, Diabetic nephropathy, Diabetic retinopathy

Introduction

Diabetes mellitus (DM) is the most common metabolic disorder, characterised by hyperglycemia, which is associated with long-term damage, dysfunction and failure of multiple organ systems, especially the kidneys, eyes, blood vessels, heart, and nerves. The prevalence of diabetes mellitus worldwide has been rising sharply in recent decades. The widespread DM and its complications have contributed tremendously to the burden of mortality and disability. Therefore, it is urgent to comprehensively understand DM pathophysiology to develop better prevention and treatment remedies.

It is reported that the total human genome is transcribed, but only 2% of them code for proteins. The vast majority of them were transcribed as non-coding RNAs which include many different subtypes, such as transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), piwi-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs) and enhancer-like RNAs (eRNAs) [1]. These non-coding RNAs are arbitrarily fallen into two major categories according to their size: small non-coding RNAs and long non-coding RNAs (lncRNAs). The non-coding RNAs containing longer than 200 nucleotides are defined as IncRNAs, and the remaining ones are considered small non-coding RNAs. LncRNAs are involved in a variety of biological processes, such as chromosome imprinting, epigenetic regulation, cell proliferation, cell cycle, metabolism, cell apoptosis, reprogramming of induced pluripotent stem cells, etc [2]. Mounting evidence indicates that lncRNAs play key roles in diabetes and diabetic complications. In this article, we will review the roles of lncRNAs in diabetic complications, and the potential therapeutic effect of lncRNAs in diabetes.

Classification and Functions of IncRNAs

LncRNAs have displayed features that can be described as ‘three more’, namely more types, more patterns and more quantity. LncRNAs can be further classified
into five categories on the basis of genomic location and sequence orientation to protein-coding genes: (1) sense IncRNAs, which are transcribed from the sense strand of protein-coding genes; (2) antisense IncRNAs, which are transcribed from the antisense strand of protein-coding genes; (3) bidirectional IncRNAs, which arise from both sense and antisense directions of transcription start areas; (4) intronic IncRNAs, which are from the introns of protein-coding genes; (5) intergenic IncRNAs, which are transcribed from intergenic regions (Fig. 1). The expression of IncRNAs is lower than protein-coding gene. Like protein-coding genes, IncRNAs exhibit strikingly cell and tissue-specific expression [3]. Accumulated evidence has proved that the cell and tissue specificity of IncRNA is directly related to chromatin state, including the cross-talk between DNA methylation and histone modification [4, 5]. DNA methylation occurs almost exclusively in CpG islands in promoter regions with the cytosine base being most frequently methylated to give rise to 5-methyl cytosine through DNA methyltransferase. Hypermethylation of these CpG islands represses gene expression, whereas hypomethylation of CpG islands activates gene expression. Histones are subject to modifications such as acetylation and methylation, which can regulate the expression of IncRNA. A number of IncRNAs are transcriptionally regulated by key transcription factors, such as p53, NFκB, Sox2, Oct4 and Nanog [6]. IncRNAs share the common key transcription factors with protein-coding genes. In addition, IncRNA possess unique expression manners different from protein-coding genes, because unsynchronized expression between protein-coding genes and their location associated non-coding genes always happens. The promoter-associated ncRNAs (pancRNAs) transcribed from the upstream regions of the transcription start site (TSS) of protein-coding genes or non-coding genes. These pancRNAs activated the expression of the adjacent protein-coding genes via sequence-specific DNA demethylation [7]. Considering that CpG islands in promoter regions tend to show bidirectional promoter activity, it is possible that there are several signature sequences in the CpG islands that direct the expression of pancRNAs [8]. Most IncRNAs execute their functions as signals, decoys, guides, or scaffolds [9] (Fig. 2). Unlike mRNA structural features, the primary sequence of IncRNA was reported to show little conservation among species, but the secondary and tertiary structures show high conservation and play a significant regulatory function in gene expression [10].

LncRNAs can regulate gene expression at the level of epigenetic regulation, transcription and post-transcriptional processing. Epigenetic regulation is the process of gene expression without changes in DNA sequences, which include DNA methylation, histone modifications and chromatin remodeling [11]. Although the diverse functions of IncRNAs are only beginning to be discovered, their potential ability to interact with and modulate the chromatin regulatory complexes is an important component of epigenetic phenomena [12]. Significant evidence has shown that numerous IncRNAs can recruit chromatin modifying complexes to specific genomic loci to regulate protein-coding gene expression (Fig. 3A). Recently, it has been reported that many IncRNAs bind to protein complexes of the Trithorax group (TrxG) or Polycomb group (PcG) families [13]. For example, polycomb repressive complexes 2 (PRC2), as a member of PcG families, are major points of IncRNA-mediated chromatin-modifying complexes. More than 20% of human IncRNAs are associated with PRC2 [13]. PRC2 is classically composed of four core components: Enhancer of zeste (EZH), Embryonic ectoderm development (Eed), Suppressor of zeste 12 homolog (Suz12) and RbAp46/48 [14]. A large proportion of IncRNAs have important roles in transcriptional regulation. LncRNAs are classified into two groups based on their effects exerted on DNA sequences: cis-acting IncRNAs and trans-acting IncRNAs (Fig. 3B). Cis-acting IncRNAs silence or activate the expression of genes in close genomic proximity, while trans-acting IncRNAs regulate chromatin states and gene expression at regions distant from their transcription site [15]. An important

Fig. 1  The genomic location of IncRNAs (shown in blue). Schematic diagram illustrates the categories of IncRNA by their genomic location and sequence orientation to nearby protein coding genes. LncRNAs can be classified into five categories: sense, antisense, bidirectional, intronic, intergenic IncRNAs.
post-transcriptional regulation mechanism of lncRNAs is their activity as microRNA sponges (Fig. 3C). MicroRNA can directly bind to the 3’ untranslated region of their target mRNAs which result in target gene silencing [16]. MicroRNA sponges can sequester the activity of the bound miRNA, thus protecting their target RNAs from repression [17]. Recent studies report natural microRNA sponges, termed competing endogenous RNAs (ceRNAs) [18]. LncRNAs are known to act as a ceRNA, which can sequester and compete with miRNA by sharing common miRNA response elements (MREs) to prevent miRNA from binding to their targets [19].

**LncRNAs and Diabetes**

Excessive hepatic glucose production and insulin resistance are the major contributing factor in T2DM. Once hepatic glucose homeostasis is disrupted, increased gluconeogenesis contributes to both fasting and post-prandial elevated blood glucose levels [20]. Additional insulin is secreted to control the elevated glucose levels, further promoting insulin resistance [21]. Forkhead box protein 01 (FoxO1) expression is a key transcription factor in regulating hepatic gluconeogenesis and insulin resistance [22], which is expressed abundantly in the liver. Increased FoxO1 activity promoted the transcription of key gluconeogenic enzymes, such as glucose-6-phosphatase catalytic subunit (G6pc) and phosphoenolpyruvate carboxykinase (Pepck) that resulted in hyperglycaemia-associated insulin resistance [23]. Recent studies have shown that some lncRNAs are involved in hepatic insulin resistance through regulating hepatic FoxO1 expression and activity. For example, upregulation of LncRNA MEG3 has been shown to increase the rate of gluconeogenesis and impair insulin-stimulated glycogen synthesis via increasing FoxO1 expression in primary hepatocytes, both of which promote hepatic insulin resistance [24]. In a recent study, it was further identified that MEG3 served as a ceRNA of miR-214 to facilitate ATF4 expression, resulting in the promotion of FoxO1 expression in primary hepatocytes and the liver tissues of HFD-fed mice [25]. In another study, the increase in lncRNA Gomafu expression also leads to elevation of FoxO1 expression by sponging miR-139, and finally to inappropriate activation of gluconeogenesis [26].

Islet β cells are solely responsible for the transcription, synthesis, and release of insulin, which are capable of regulating glucose homeostasis. The loss or impairment of islet β cell function appears to underlie the pathogenesis of diabetes mellitus. Recently, the potential contribution of lncRNAs to regulating β cell function has been investigated. Three β cell-specific transcriptional regulators, Pdx-1 (pancreatic and duodenal homeobox-1), NeuroD1 (neurogenic differentiation 1) and MafA (V-maf musculoaponeurotic fibrosarcoma oncogene homo-
logue A), have been demonstrated to play a crucial role in regulating pancreas development and β cell differentiation, which stimulate insulin gene expression in response to elevated blood glucose [27]. For example, lncRNA Meg3 and PLUTO have been identified to be novel regulator of insulin synthesis and secretion in pancreatic β cell [28, 29]. In the recent study, Meg3 expression was relatively higher in mouse pancreas than any other organs including liver, spleen, lung and kidney. Suppression of Meg3 expression in vitro and knockdown of Meg3 in vivo could affect insulin synthesis and secretion by decreasing the expression of Pdx-1 and MafA [28]. The different role in pancreas and liver maybe involves in tissue distribution and different stage of diabetes. In human, lncRNA PLUTO is downregulated in islets from donors with type 2 diabetes or impaired glucose tolerance through affecting the expression of PDX1 [29].

Fig. 4 summarized the possible mechanism of lncRNA in T2DM.

The Possible Mechanism of lncRNA in Diabetic Nephropathy (DN)

DN is the leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) [30]. It has a poor prognosis, speedily developing into renal dysfunction and uremia, and is thus a major cause of death and disability in DM. Emerging evidence has shown that lncRNAs play a crucial role in the development of DN.

LncRNAs associate with glomerular injury

The pathogenesis of DN is related to the excessive proliferation and fibrosis of mesangial cells (MCs) [31]. Several lncRNAs have been proved to contribute to regulate MCs proliferation and fibrosis in vivo and in vitro. For example, lncRNA CYP4B1-PS1-001 was significantly downregulated in vitro and in vivo in early diabetic nephropathy. This study also indicated that CYP4B1-PS1-001 overexpression inhibited MCs proliferation and fibrosis [32]. Wang S further identified that
CYP4B1-PS1-001 overexpression could regulate the ubiquitination and degradation of nucleolin to inhibit MCs proliferation and fibrosis [33]. Recent reports have also demonstrated that lncRNA NEAT1 (nuclear enriched abundant transcript 1) and 1500026H17Rik (150Rik) were significantly upregulated in renal tissue of diabetic animal model and in MCs [34, 35]. Another study shows that Gm4419 could activate the NF-κB pathway and NACHT, LRR and PYD domain-containing protein 3 (NLRP3) inflammasome in MCs. Meanwhile, Gm4419 knockdown could obviously inhibit the expressions of pro-inflammatory cytokines and renal fibrosis biomarkers, and reduce cell proliferation in MCs under high-glucose condition. This finding suggests that PVT1 may contribute to the excessive accumulation of ECM in DN [40]. In addition, recent data identified that Danggui Buxue Tang (DBT), a traditional Chinese medicine decoction, could attenuate MCs excessive proliferation and ECM accumulation through downregulating PVT1 [41]. LncRNA TUG1 (taurine upregulated gene 1) also has been considered to be as a novel regulator involving in ECM accumulation in DN. LncRNA TUG1 acts as an endogenous sponge of miR-377, downregulating miR-377 expression levels and relieving the inhibition of its target gene PPARγ, thus attenuating HG-induced MCs proliferation and ECM accumulation [42].

Fig. 4 Long noncoding RNAs (lncRNAs) associated with DM.

Adult podocytes are unique terminally differentiated glomerular epithelial cells and a key component of the glomerular filtration barrier [43]. Loss of podocyte leads to proteinuria, and glomerulosclerosis in DN [44]. Mitochondrial dysfunction is involved in podocyte injury [45] and is central to the pathogenesis of DN [46]. The expression of PPARγ coactivator 1α (PGC-1α) has gained attention as a key mediator of mitochondrial...
bioenergetics and progression of DN [47]. LncRNA TUG1 was significantly repressed in the podocytes of diabetic mice. Podocyte-specific overexpression TUG1 could improve mitochondrial bioenergetics through increasing PGC-1α expression, along with improvements in several key features of DN [48]. Summing up the above studies, TUG1 plays a very important role in attenuating MCs proliferation, ECM accumulation and podocyte injury. Bai X et al. used renal biopsy tissues from DN patients and analyzed the lncRNA expression profiles to overcome the conundrums of species differences. They found that renal LINC01619 was shown to be markedly downregulated in DN patients, which is associated with proteinuria and declined renal function. They used HG cultured podocytes and diabetic rats to further explore the molecular mechanism of LINC01619 in DN. LINC01619 played biological function by serving as a molecular sponge for miR-27a in DN. LINC01619 upregulated FOXO1 expression by rescuing the inhibitory effect of miR-27a on FOXO1, thus alleviating oxidative stress and podocyte injury [49]. LncRNA MALAT1 (metastasis associated lung adenocarcinoma transcript 1) was also remarkably up-regulated in the kidney cortices of diabetic mice with a radical impairment of glomerular podocytes and marked proteinuria. Early interference of MALAT1 showed a protective effect on functional integrity of podocytes in vitro [50].

LncRNAs associate with tubular injury

Tubular injury is also widely considered as a critical pathological feature of DN [51]. LncRNA MALAT1 was up-regulated in kidney tissues of diabetic mice and in HK-2 cells, which promotes HK-2 cells injury and exerts a critical impact on DN. MALAT1 is acting as an endogenous sponge of miR-23c, downregulating miR-23c expression levels, and ultimately leading to cell pyroptosis under hyperglycemic conditions both in vitro (in HG-treated HK-2 cells) and in vivo (STZ-induced diabetic rats) [52].

Clinical trials

The effect of lncRNA in renal tissues of diabetic animal models and various kidney cells has been demonstrated. However, expression of lncRNA in human renal tissues of DN remains unknown. Several clinical trials have confirmed the effect of lncRNAs in DN. For example, it has been proved that lncRNA MALAT1 was up-regulated in kidney tissues of diabetic animal model and in cultured cells. The serum MALAT1 was also overexpressed in diabetic ESRD patients [53]. Although this study is limited by its relatively small sample size and only the patients with ESRD, their functional role could provide a comprehensive view of the pathogenesis of DN. In T2DM patients with chronic renal failure, lncRNA CASC2 in serum and renal tissue is downregulated, compared with other diabetes-related complications. So, CASC2 has the prospect of becoming a diagnostic biomarker for DM-induced chronic renal failure [54].

Fig. 5 summarized the possible mechanism of lncRNA in diabetic nephropathy.
The Possible Mechanism of lncRNA in Diabetic Retinopathy (DR)

DR is the most common vision-threatening complication that may eventually lead to blindness in DM [55]. Recent studies showed that lncRNA participated in the development of DR.

lncRNAs associate with aberrant neovascularization

In DR, aberrant neovascularization leads to the formation of highly permeable blood vessels across the retina. Vascular endothelial growth factor (VEGF), which promotes angiogenesis and increases vascular permeability, is closely correlated with the development and progression of DR [56]. LncRNA ANRIL knockdown could downregulate VEGF-induced retinal microvascular permeability in human retinal endothelial cells (HRECs) and the ANRILKO mice. LncRNA ANRIL mediated VEGF upregulation through the PRC2 complex and the histone acetylator p300 [57]. Another study also demonstrated that lncRNA MIAT expression was significantly higher upon high glucose stress in vivo and in vitro. MIAT knockdown could alleviate diabetes-induced retinal vascular leakage and ameliorate visual function through decreasing VEGF-induced tube formation. During angiogenesis, lncRNA-MIAT functions as a ceRNA to regulate VEGF levels by sponging miR-150-5p in retinal endothelial cells [58].

Inflammation is also an important factor contributing to the pathogenesis of DR [59]. MALAT1 is capable of affecting vascular leakage in the diabetic retina through regulating the expressions of inflammatory transcripts. In HRECs, the expressions of MALAT1 and common inflammatory markers (IL-6, TNF-α, MCP-1 and IL-1β) peaked at 48 hours. Moreover, it was further demonstrated that MALAT1 knockdown dramatically reduced the expression of overall inflammatory markers in vitro. In diabetic MALAT1 KO mice model, MALAT1 knock-out similarly diminished vascular leakage in the diabetic retina and alleviated diabetes-induced retinal inflammatory cytokines. Overall, MALAT1 is capable of impacting the expressions of inflammatory transcripts through its association with components of the PRC2 complex in diabetes [60].

Several lncRNAs, such as lncRNA H19, MIAT, MEG3, were found to be significantly associated with the development of DR by regulating TGF-β1 and its signalling pathways [61-63]. For example, LncRNA H19 expression level was significantly lower in HRECs after glucose exposure and in the vitreous humour of diabetic participants. Overexpression of H19 reversed endothelial-mesenchymal transition markers by suppressing TGF-β1 [61]. LncRNA MEG3 and MIAT also were reported to influence DR through TGF-β1 in HRECs and in diabetic patients [62, 63].

lncRNAs associate with neuronal dysfunction

Recently, it has been gradually recognized that neuronal dysfunction plays an important role in the pathogenesis of DR [64]. Retinal ganglion cell (RGC) injury and glial cells activation are the important pathological features of diabetes-induced retinal neurodegeneration. SOX2OT knockdown could ameliorate visual function and protect RGCs against injury through regulating NRF2/HO-1 signaling in the retinas of STZ-induced diabetic mice and the RGCs under high glucose environment [65]. RNC3 is found to be significantly up-regulated in diabetic retinas and high glucose-treated retinal endothelial cells [66]. RNC3 knockdown could inhibit retinal reactive gliosis and alleviate diabetes mellitus-induced retinal neurodegeneration in vivo [67].

Fig. 6 summarized the possible mechanism of lncRNA in DR.

Conclusion

At present, more and more lncRNAs are identified, yet their physiological and pathological functions are still in its infancy. A number of recent studies have focused on the functions and working mechanisms of lncRNAs in diabetes and its microvascular complications. The ultimate goal of basic research is to solve problems encountered in clinical work. Through studying the mechanism of lncRNA in DM and diabetic microvascular complications, we can silence or active the lncRNAs via exogenous means (e.g., gene knock in, RNA interference and gene supplement) to provide a new prevention and treatment method. However, the mechanism of lncRNA involved in the pathogenesis of DM is quite complicated. Further investigations are essential to improve our understanding regarding the biological functions and molecular characteristics of lncRNAs in diabetes and its complication. These new advancements suggest that lncRNAs could have a promise to serve as new diagnostic markers and novel therapeutic targets for diabetes.

Declaration of Interest

The authors declare no conflict of interest.
Fig. 6  Long noncoding RNAs (lncRNAs) associated with DR.

References

LncRNAs in diabetic microangiopathy


