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Circulating Cell-Free lncRNAs and Their Potential as Epigenetic Biomarkers for Type 2 Diabetes Mellitus

Prof. Rowyda Nawwaf Al-Harithy

[1] King Abdulaziz University (KAU)

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ABSTRACT

Type 2 Diabetes Mellitus (T2DM) is one of the fastest-growing metabolic diseases worldwide, primarily affecting adults, with most diagnoses occurring in individuals over 45. As a polygenic disorder, it is influenced by a complex interaction of environmental, genetic, and epigenetic factors. These factors contribute to its pathophysiology, including insulin resistance, primary β-cell failure, or a combination of both, often associated with oxidative stress. Type 2 Diabetes Mellitus frequently remains undiagnosed for years as patients progress through the prediabetes stage, characterized by hyperglycemia without noticeable symptoms. Moreover, a definitive cure remains elusive. Given the significant global health threat posed by Type 2 Diabetes Mellitus, there is an urgent need to advance research into noninvasive biomarkers for early diagnosis, prognosis, and the prediction of therapeutic responses. Recently, non-coding RNAs (ncRNAs) have attracted considerable attention. Although these molecules do not encode proteins, they play pivotal roles in various cellular processes, and dysregulation of their expression is increasingly linked to diseases. Aberrant ncRNA profiles have been identified in many diabetic patients, particularly those with complications. Recent evidence indicates that epigenetic dysregulation is a key driver in the onset and progression of Type 2 Diabetes Mellitus. These findings highlight non-coding RNAs as crucial players in developing Type 2 Diabetes Mellitus, with significant potential as biomarkers for predicting and monitoring disease progression. Early identification of diabetes or prediabetes can mitigate the risk of long-term complications, including cardiovascular disease, retinopathy, neuropathy, and nephropathy. This chapter focuses on circulating cell-free long non-coding RNAs, specifically linear and circular RNAs, and their roles in developing Type 2 Diabetes Mellitus. These molecules are particularly intriguing due to their unique structural properties and ability to regulate diverse biological processes. As research into circulating cell-free lncRNAs continues to expand, these molecules hold promise for providing novel insights into the disease's molecular mechanisms, offering new possibilities for early diagnosis, prognosis, and personalized treatment strategies.

Keywords: Epigenetic biomarkers, Ccf-lncRNAs, LncRNAs, CircRNAs, Type 2 Diabetes Mellitus.

1. Introduction

Epigenetic mechanisms constitute dynamic modes of gene regulation, influencing both transcription and subsequent gene expression. These heritable yet reversible processes, including DNA methylation, histone modifications, chromatin remodeling, and non-coding RNA activity, play crucial roles in the development and progression of metabolic diseases [1]. Research indicates that epigenetic changes are vital for cellular adaptation, acting as key regulators in various physiological and pathological processes, including aging [2]. These modifications allow cells to respond to environmental cues without altering their DNA sequence. As a result, epigenetic changes are increasingly recognized as valuable biomarkers with significant potential for early disease detection, prognostic evaluation, and treatment monitoring. The discovery of disease-specific epigenetic patterns across numerous human disorders underscores their utility as indicators of pathological predispositions [3]. Biomarkers are becoming increasingly valuable tools in drug development and disease research, serving as early warning systems for disease risk, deepening our understanding of disease origins and progression, and enabling the monitoring of treatment effectiveness [4]. Identifying changes in the epigenetic landscape associated with human diseases and the factors driving these changes opens new avenues for discovering additional epigenetic biomarkers. While research on epigenetic biomarkers and their role in metabolic diseases is still in its early stages, it is advancing rapidly [5]. Progress in chemical and biological methods to detect specific changes within genomes and transcriptomes is fueled by a growing interest in understanding DNA and RNA modifications and their associated molecular mechanisms. As the number of epigenetic biomarker candidates continues to grow, the opportunity to integrate these biomarkers into practice has never been more promising. However, realizing this potential requires the development of robust and reliable methods for detecting and analyzing epigenetic modifications. These advancements will help facilitate the smooth transition from research laboratories to routine clinical practice [6].

Among these biomarkers, long non-coding RNAs (lncRNAs) have gained attention as sensitive, non-invasive tools for diagnosis, prognosis, and prediction of therapeutic responses. Research has highlighted the importance of the structural characteristics of lncRNAs in determining their functional interactions with other macromolecules. The primary, secondary, and tertiary structures of lncRNAs are intricately linked to their context-specific roles, emphasizing their versatility and complexity in biological processes [7]. These complex structures allow lncRNA to interact with other molecules, including proteins, DNA, mRNAs, and other non-coding RNAs, such as microRNAs and small interfering RNAs, further emphasizing their critical roles in cellular regulation and biological function.

A subset of lncRNAs found in extracellular body fluids, such as urine, plasma, serum, and saliva, is known as circulating cell-free lncRNAs (ccf-RNAs). These molecules are released into circulation through various mechanisms, including apoptosis, necrosis, and active secretion via exosomes [8,9]. Their remarkable stability in bodily fluids, with advancements in detection methods, underscores their potential for widespread clinical applications[10,11]. They also represent a diverse family of biomolecules released into the bloodstream from various tissues. Their presence in circulation reflects physiological and pathological states, making them valuable for therapeutic monitoring and diagnostics [12-14]. The growing interest in ccf-lncRNAs stems from their unique attributes, including stability, accessibility, and strong associations with numerous diseases. These features position ccf-lncRNAs as powerful tools in advancing precision medicine, spanning early diagnosis and the development of novel therapies. As research progresses, ccf-lncRNAs are emerging as a focal point in molecular biology and medicine, with ongoing studies aiming at uncovering their functions and potential clinical applications [15].

Recent investigations have demonstrated that specific ccf-lncRNAs are linked to the pathogenesis of Type 2 Diabetes Mellitus (T2DM) [16-18]. These RNAs present an exciting opportunity to explore the molecular mechanisms underlying T2DM. However, the specific roles of ccf-lncRNAs in T2DM remain incompletely understood. Further research into the functions of ccf-lncRNAs, especially those that outline the disease's epigenetic basis in T2DM, will significantly enhance the field of precision medicine. Such advancements can potentially transform biomedicine and clinical diagnostics by enabling earlier intervention, improved prevention, and more targeted management of T2DM [19, 20].

2. Type 2 Diabetes Mellitus

This metabolic disorder is the primary contributor to diabetes prevalence worldwide, accounting for over 96% of all diabetes cases in 2021 [21]. This condition poses a significant clinical and public health burden due to its impact on the body's glucose regulation system. Uncontrolled T2DM results in persistently high blood sugar levels, ultimately leading to complications such as nephropathy, retinopathy, and neuropathy, each difficult to treat [22]. Globally, T2DM is recognized as one of the top 10 causes of death [23]. Individuals with T2DM often experience a reduced quality of life and diminished functional capacity, increasing their risk of severe diseases and premature mortality [24]. While T2DM affects both men and women equally, men are typically diagnosed at a younger age and tend to have a lower body fat percentage than women [25]. Over the past few decades, the prevalence of diabetes has risen steadily. According to the 2023 World Health Organization (WHO) report, diabetes causes 1.5 million deaths annually and affects approximately 422 million people globally. By 2050, projections estimate that over 1.31 billion individuals will be living with diabetes [26]. An effective prevention strategy hinges on identifying individuals most vulnerable to T2DM [27, 28]. Prompt diagnosis would enable the implementation of preventative measures, slowing disease progression and reducing the likelihood of complications that are often more challenging to manage.

Type 2 Diabetes Mellitus is a multifactorial and progressive illness arising from a complex interplay of pathological processes and molecular pathways; many

are cell-type-specific[29]. Understanding these mechanisms is crucial for developing targeted strategies to address this global health challenge. Decades ago, the prevailing understanding of T2DM pathophysiology centered on insulin resistance as the primary abnormality. β-cell dysfunction was considered a secondary phenomenon, arising as β-cells became "exhausted" and unable to produce sufficient insulin to compensate for the increasing resistance [30-34]. This perspective also emphasized the crucial role of adipose tissue in T2DM development and progression, particularly through its involvement in insulin resistance and metabolic dysfunction [35,36]. Conversely, an alternative hypothesis suggested that β-cell dysfunction precedes the onset of dysglycemia [37-39]. According to his theory, β-cell dysfunction, characterized by reduced insulin production, is a fundamental defect independent of insulin resistance and typically arises early in the etiology of dysglycemia [40]. In T2DM, β-cell failure has been associated with multiple mechanisms that affect β-cell differentiation, proliferation, insulin production, and cell survival [41- 43]. These findings validate the respective roles of insulin resistance and β-cell malfunction in the pathogenesis of T2DM [44]. Moreover, β-cell failure is exacerbated by several factors, including glucotoxicity, endoplasmic reticulum (ER) stress, mitochondrial dysfunction, inflammation, metabolic (lipid) signaling, and other variables. These factors create a vicious cycle that perpetuates β-cell deterioration. Notably, abnormal micronutrient levels have been shown to influence many of these pathways, further compounding β-cell dysfunction [45].

Although lifestyle and environmental factors are well-established risk factors for T2DM, heritability is estimated to account for 69% of the risk. Genome-wide association studies (GWAS) have identified over 700 independent genetic loci associated with an increased risk of T2DM. Many of these genes are believed to play critical roles in the formation, function, or mass regulation of β-cells [46-50]. The development of T2DM ultimately requires a combination of impaired β-cell activity and increasing insulin resistance. This dual dysfunction stems from localized abnormalities within individual tissues and systemic dysregulation of inter-tissue communication[51]. Crosstalk among adipose tissue, liver, skeletal muscle, pancreas, and intestine is central to the pathogenesis of insulin resistance and β-cells dysfunction. While the role of endocrine dysregulation in T2DM pathogenesis is well-recognized, further research is essential to unravel the complex progression from normoglycemia to hyperglycemia. Investigating inter-organ interactions through multi-omics approaches has highlighted potential mediators of crosstalk, such as ccf-lncRNAs, which may improve the ability to predict T2DM risk.

Recent studies have also emphasized the significance of epigenetic changes and markers in the onset of T2DM. While genetic loci contribute to T2DM risk, epigenetic biomarkers play a more prominent role by coordinating gene expression within a broad regulatory network rather than acting independently. This highlights the potential for epigenetic insights to revolutionize our understanding and management of T2DM.

3. Non-coding RNAs

Functional ncRNAs represent a diverse group of heterogeneous transcripts derived from genes that do not encode proteins. A substantial portion of eukaryotic genomes comprises genes that code for ncRNA molecules. It is well known that 98–99% of the human genome does not encode proteins; yet, this vast non-coding region is transcriptionally active, producing a broad spectrum of ncRNAs with complex regulatory and structural functions[52-54]. Despite their importance, ncRNAs were initially called for what they are not rather than what they are. This stems from the earlier perception of RNA as merely an intermediary between genes and proteins, with other housekeeping non-coding RNAs like ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), and other small nuclear RNAs (snRNAs) serving as secondary to the function. However, it was widely accepted by the early 21st century that ncRNAs are regulatory molecules with distinct biogenesis and genomic origins compared to mRNAs[55]. They have been identified as important participants in biological control and have received considerable attention over the last 44 years. In various tissues and cells, they manifest in distinct ways. They also regulate multiple cell types and tissues, including pluripotency, imprinting, transcription, splicing, translation, and cell differentiation and development[56]. Given their critical role in maintaining cellular functionality, the disruptions in ncRNAs' expression profiles are linked to the development of various illnesses[57,58]. Research into the non-coding genome has greatly advanced our understanding of the multi-level complexity of the human genome and opened new avenues for therapeutic innovation.

The discovery and characterization of the first regulatory ncRNA gene, *micF* RNA, occurred in prokaryotes during the 1980s[59-62]. Before this breakthrough, RNAs were largely viewed as inert polymers supporting protein production, and the idea of RNAs as regulators was completely foreign to science. By the 1990s, ncRNAs were identified in most eukaryotic organisms. A few lncRNAs, such as *H19* and *Xist*, were characterized during the pre-genomic era, but these remained exceptions until the ENCODE (Encyclopedia of DNA Elements) project in 2005. This initiative revealed that up to 80% of the human genome can be transcribed into ncRNAs[63, 64]. However, the biogenesis, size, and function of the ncRNAs family are heterogeneous; they are categorized based on either their function or their nucleotide length[65]. By length, ncRNAs are divided into two main groups. The categories include microRNAs (miRNAs), short nucleotides less than 200 bases in length, and nucleotides longer than 200 bases, including linear ncRNAs and circular RNAs (circRNAs), which differ significantly in their origin and structure[66].

4. Linear lncRNAs

The lncRNAs represent a crucial class of molecules, with key members playing essential roles in maintaining genomic integrity and regulating epigenetic processes[67]. They are considered a subclass of transcripts with short open reading frames (sORFs) that exhibit translation signatures. These sORFs encode small proteins, often known as micro-peptides, which contribute to protein translation[68]. The properties of lncRNAs suggest that

mRNA translation is more extensive and complex than previously understood. They represent a highly diverse class of genes that perform various molecular functions essential for biological processes. Among these functions, lncRNAs regulate gene transcription by interacting with chromatin-modifying enzymes, highlighting their significance in numerous cellular processes. Acting at transcriptional, post-transcriptional, and structural levels, lncRNAs serve as master regulators of gene expression[69]. Their gene regulatory activities influence key physiological processes, including cell differentiation, growth, cellular responses to stress and external stimuli, as well as the functioning of neurological and muscular systems[70,71]. They also play critical roles in cardiovascular health[72], adipose tissue regulation [73], and the hematopoietic and immune systems. Dysregulation of lncRNAs has been implicated in various pathologies, emphasizing their crucial roles in maintaining health and contributing to disease development[73,74].

Initially, lncRNAs were identified as mRNA-like transcripts that do not encode proteins. Later studies uncovered additional features distinguishing lncRNAs from mRNAs. LncRNAs constitute a significant portion of the genomes of complex species, and their number in humans continues to grow as research advances. Recent estimates suggest the number of lncRNA genes in humans exceeds 95,000, reflecting their complexity[75,76]. This complexity arises from their rapid diversification, cell-specific expression patterns, and ongoing discovery of new variants. They are also localized to distinct subcellular sites, primarily the nucleus and cytoplasm[77]. Subcellular fractionation techniques have revealed that, in some cell types, a significant portion of lncRNAs is exported to the cytoplasm, where cytoplasmic lncRNAs appear to outnumber nuclear-enriched ones[78]. Moreover, growing evidence has demonstrated the presence of mitochondria-encoded lncRNA (mt-lncRNAs) transcribed from mitochondrial DNA (mtDNA)[79]. These mt-lncRNAs are implicated in various biological processes[80]. Over the past decade, substantial progress has illuminated the biogenesis of lncRNAs and their distinct functions[81]. Based on their roles, lncRNAs can be categorized into three subtypes: non-functional lncRNAs, which are merely transcriptional byproducts; lncRNAs with self-sufficient transcription; and third functional lncRNAs that operate in cis and/or trans orientations[82]. The unique subcellular localizations are closely linked to their distinct biogenesis and functions. In the nucleus, lncRNAs perform critical roles, including transcriptional regulation, nuclear structure organization, RNA processing, and splicing regulation. They also act as decoys, enhancers, and scaffolds[83]. In the cytoplasm, lncRNAs regulate gene expression post-transcriptionally by stabilizing mRNAs, modulating translation, acting as miRNA sponges or decoys, preventing protein degradation, altering signal transduction pathways, and directing mRNAs to specific subcellular compartments[84-86]. The lncRNAs' roles are also informed by their interactions with proteins, mRNA, DNA, and other ncRNAs. While some lncRNAs have well-defined roles, the functions of many remain unclear. LncRNAs regulate the expression of nearby genes, influence transcription, and play roles in DNA replication, damage response, repair, and chromatin biology. Additionally, lncRNAs are involved in the mRNA life cycle, including splicing, turnover, translation, and signaling pathways. Increasing evidence indicates that lncRNAs work collaboratively to regulate gene expression, forming extensive regulatory networks.

The complex functions of lncRNAs, their numerous isoforms, and their interactions with other genes make their classification and annotation particularly challenging. They can be categorized based on length, function, location, and targeting mechanism, though there is no unified standard for their classification. Based on their genomic position relative to protein-coding genes, lncRNAs can be classified as sense, antisense, bidirectional, intronic, intergenic, or enhancer lncRNAs[87]. Additionally, they are categorized by their modes of action as bait, scaffold, signal, or guide lncRNAs[88]. Subtypes of lncRNAs are further classified by chromosomal location, function, and structural characteristics, including antisense, sense, intronic, bidirectional, and intergenic lncRNAs[89]. Compared to mRNAs, lncRNAs splice less efficiently and produce fewer spliced transcripts[90,91]. They are more prevalent and evolve rapidly due to their structure-function relationships than protein-coding transcripts. Most lncRNAs feature a 5′ cap and a poly(A) tail at the 3′ ends, suggesting similarities in transcription mechanisms with mRNAs[92]. Alternative splicing expands the transcriptome for both mRNAs and lncRNAs. They typically exhibit more tightly regulated expression patterns and are less abundant than protein-coding genes [93,94]. The expression is highly cell-specific, aligning with their roles in defining cell state and developmental trajectory[95]. In contrast to mRNA sequences encoding the proteome, lncRNAs are less conserved across species[96,97]. However, loci expressing lncRNAs share many characteristics with protein-coding genes, including promoters, multiple exons, alternative splicing, distinctive chromatin signatures, and varying half-lives[98,99]. Many lncRNAs are spliced and polyadenylated, earning them the designation "mRNA-like." However, some lack polyadenylation or 7-methylguanosine caps[100]. LncRNAs are primarily transcribed by RNA Polymerase II (Pol II), though RNA Polymerase III also transcribes them at some loci[101]. Due to weak internal splicing signals and the long distance between the 3' splice site and the junction, lncRNAs are spliced less efficiently than the mRNA[102]. Additionally, ncRNAs lack signature motifs of mRNAs, such as the Kozak consensus sequence and long open reading frames (ORFs)[103]. Long non-coding RNAs are expressed differently in various tissues and cell types at different stages of development and are often dysregulated in a disease-specific manner[104]. Ccf-lncRNAs are emerging as ideal noninvasive epigenetic biomarkers in diverse clinical settings, as they carry real-time information[105-107]. Upon release, lncRNAs bind to RNA-binding proteins and are encapsulated within exosomes or apoptotic bodies[108,109]. Since the early 2000s., lncRNAs have been implicated in human disorders, with increasing recognition of their significance in diseases such as cancer, obesity, and cardiovascular disease.

5. Circular RNAs

Eukaryotic circRNAs are abundant in exosomes, regulate gene expression, and exhibit tissue- and cell-specific expression patterns [110,111]. These naturally occurring

biomolecules are derived from nucleolar precursor messenger RNA (pre-mRNA) and possess a covalently closed loop structure[112]. They are widely expressed in human cells, with their levels often exceeding those of their linear isomers by more than tenfold[113]. More than 90% of circRNAs are derived from protein-coding exons, while smaller fractions originate from intronic regions[114]. The circRNAs have longer half-lives than their linear counterparts because they lack a 5' cap and a 3' poly(A) tail, rendering them resistant to RNase-mediated degradation[115]. Unlike linear mRNAs, circRNAs are predominantly localized in the cytoplasm; human circRNAs can be released from cells or transported between the cytoplasm and the nucleus. In the nucleus, circRNAs can enhance the transcription of their parent genes. The unique structure and exceptional stability of circRNAs confer distinct regulatory capabilities, differentiating them from linear counterparts in biological functions and potential applications. CircRNAs have long been overlooked despite their growing significance, and their full regulatory potential remains incompletely understood.

The circRNAs, first described in 1976 in the *Sendai* virus, were thought to be rare splicing errors [116,117]. However, with advancements in bioinformatics and high-throughput sequencing, it has become clear that circRNAs are highly conserved and widely expressed across species[118,119]. Bioinformatic and experimental data suggest that circRNAs are formed based on a common RNA motif, which includes a 7-nucleotide GU-rich section near the 5' splice site and an 11-nucleotide C-rich section near the branch point[120]. Recent research has further clarified the processes involved in circRNA production, revealing that circRNAS can originate from exons, introns, or a combination of both[121]. Based on their structure, they are classified into three main types: exon-intron circRNAs (ElciRNAs), intronic circRNAs (ciRNAs), and exonic circRNAs (ecircRNAs). The circularization process of circRNAs can be facilitated by RNA-binding proteins (RBPs) and the spliceosome, with mechanisms depending on reverse complementary motifs and lariat-mediated circularization[122,123]. Once formed, circRNAs are typically localized to the nucleus or cytoplasm and may also be released from the cells. However, recent research has shown that circRNAs can also be found in mitochondria[124]. Depending on their origin and location, circRNAs produced by the nuclear genome are referred to as nuc-circRNA. CircRNAs associated with mitochondria can be classified into three groups based on their origin and location: (1) circRNAs encoded by the mitochondrial genome and situated within the mitochondria; (2) circRNAs encoded by the mitochondrial genome and situated in the cytoplasm or secreted from cells; and (3) circRNAs encoded by the nuclear genome but localized in the mitochondria[125,126]. Despite these findings, much remains unknown about the biological roles and molecular mechanisms of circRNA in human mitochondria, and their significance is not yet fully understood, especially when compared to mitochondrial proteins, DNA, and miRNAs. Remarkably, circRNAs perform diverse functions in various biological processes. One primary role is acting as miRNA sponges, regulating the expression of target genes by inhibiting miRNA activity. They usually compete with their parental genes for mRNA-binding sites due to their sequences complementary to those of the parent genes[127]. A single circRNA, functioning as a competitive endogenous RNA, can regulate one or multiple miRNAs through its miRNA-binding sites. Additionally, the 3' untranslated regions or noncoding transcripts of specific genes also contain miRNA-binding sites that interact with circRNAs. Recent studies provide compelling evidence that circRNAs can be translated into proteins. They utilize the start codon of their host mRNA and rely on evolutionarily conserved termination codons within their circular open reading frames (circORFs). They undergo cap-independent translation due to their closed structure and the presence of internal ribosome entry sites (IRES), which distinguishes them from linear mRNA[128]. In addition to these roles, circRNAs act as signaling molecules, facilitating intercellular communications. By modulating miRNA levels in host cells, circRNAs are sorted into exosomes for secretion. They are also more commonly sorted into exosomes than their linear counterparts, highlighting their importance in intercellular signaling pathways[129]. Advances in RNA sequencing technology and bioinformatics have facilitated the discovery of numerous circRNAs and their diverse biological roles[130,131]. Their stability, largely due to their covalently closed structure, makes them resistant to exonuclease degradation, allowing them to persist longer in cells than linear RNAs (Table 1).

Table 1: Differences and similarities between linear lncRNAs and circRNAs

Feature	Linear lncRNAs	Circular RNAs
Structure	Linear (5' to 3') with exons and introns	Covalently closed loop, no free ends
Stability	Less stable, prone to exonuclease degradation	Highly stable, resistant to exonucleases
Biological Function	Gene regulation (transcriptional control, epigenetics)	miRNA sponge, regulation of gene expression, protein translation
Mechanism of Action	Interact with DNA, RNA, proteins, chromatin modifiers	Bind miRNAs, interact with RNA-binding proteins, affect splicing
Expression in Disease	Abnormal expression in metabolic disorders (T2DM)	Biomarkers for various diseases (T2DM)
Therapeutic Potential	Targeting for gene regulation, epigenetic therapies	Targeting for miRNA sponging, gene expression modulation
Examples relevant to T2DM	MALAT1, MEG3, H19, TUG1, lnc-GHRL-3:2, lnc-GHRL-3:3, ANRIL, NEAT1	hsa_circ_0054633, hsa_circ_0001445, hsa_circ_0000284, circANKRD36, circHIPK3

The observation that circRNA levels fluctuate with age is particularly intriguing. It suggests their expression patterns may be linked to cellular aging processes, tissue-specific regulation, and disease progression. This makes circRNAs promising candidates as biomarkers for age-related diseases, including neurodegenerative disorders, cardiovascular diseases, cancer, and diabetes mellitus[132,133]. Their involvement in these diseases underscores their potential as diagnostic and therapeutic targets, paving the way for new approaches in precision medicine.

6. Role of lncRNAs in Type 2 Diabetes Mellitus pathogenesis

In diabetes research, particularly concerning T2DM, lncRNAs have emerged as significant regulators[134]. Over the past 14 years, research on the role of lncRNAs in T2DM has expanded considerably. The first major study linking lncRNAs to T2DM was published in 2010, identifying the lncRNA *HOTAIR* (HOX transcript antisense intergenic RNA) as a key player in glucose metabolism and insulin signaling[135,136]. Subsequent research has further elucidated the relationship between lncRNAs and T2DM, uncovering their critical roles in the disease's pathophysiology. These studies have identified numerous lncRNAs that influence essential pathways, including gene regulation, insulin sensitivity, inflammation, β-cell function, epigenetic modulation, and metabolic processes. These findings underscore the potential contribution of lncRNAs to the development and progression of T2DM. For instance, lncRNA *MALAT1* (Metastasis Associated Lung Adenocarcinoma Transcript 1) regulates the expression of glucose transporters and enzymes involved in glycolysis, thereby modulating glucose uptake and utilization. lncRNA *MALAT1* also plays a significant role in the insulin signaling pathway[137]. Additionally, lncRNA *H19* influences the expression with glucose metabolism and lipid storage, while lncRNA *SRA* regulates genes associated with lipid metabolism[138]. Dysregulated expression of lncRNA *SRA* has been linked to dyslipidemia, which contributes to insulin resistance and metabolic syndrome. Another lncRNA, *GA55*, has been associated with obesity-related complications in T2DM and influences the expression of multiple genes involved in the insulin signaling pathway.

Long non-coding RNAs also impact the proliferation and function of pancreatic β-cells. Genetic studies have provided definitive evidence that lncRNAs play a role in β--cell functionality. Early GWAS studies revealed that most single nucleotide polymorphisms (SNPs) associated with T2DM are in non-protein-coding regions of the genome, highlighting the importance of lncRNAs in the disease's genetic and functional landscape[139]. LncRNA *HOTAIR*, for example, influences the survival and functionality of pancreatic β-cells. A fundamental component of T2DM is the inflammatory response and oxidative stress. lncRNA *HOTAIR* can modulate inflammatory pathways by Influencing cytokine production. Additionally, lncRNA *LINC00673*, regulates the expression of pro-inflammatory cytokines, exacerbating inflammation linked to insulin resistance. Another mechanism through which lncRNAs regulate metabolic activities is via epigenetic modifications. lncRNA *ANRIL* can act as an epigenetic regulator by altering chromatin structure and affecting gene transcription. It recruits enzymes to specific gene loci to modify chromatin without changing the DNA sequence. The lncRNAs can also function as miRNA sponging, as demonstrated by molecules like *H19* and *BGL3*. These lncRNAs bind to miRNAs, preventing them from inhibiting their target mRNAs, which significantly impact pathways involved in T2DM. Certain lncRNAs regulate mRNA stability, influencing protein translation by stabilizing or destabilizing the mRNA transcripts of genes related to glucose metabolism and insulin signaling. Furthermore, some lncRNAs, such as *MALAT1*, act as scaffolds, serving as platforms for protein complexes and facilitating the action of regulatory elements that govern gene expression.

Several lncRNAs have been shown to play crucial roles in adipogenesis and fat distribution, both of which are key factors in the pathophysiology of T2DM[140]. They can regulate the process of adipogenesis in different ways. Some lncRNAs can regulate the differentiation of preadipocytes into mature adipocytes by modulating the expression of adipogenic transcription factors like PPARγ and C/EBPα. Several others, like ADNCR (adipocyte differentiation-associated noncoding RNA), act as inhibitors of adipogenesis by interacting with specific signaling pathways or transcription factors. Others, like lncRNA H19, have been shown to promote adipogenesis by enhancing the activity of key pathways. Also, the process of fat distribution, particularly the balance between visceral and subcutaneous fat, is crucial in T2DM risk. LncRNAs can influence where fat is stored by modulating the activity of genes related to lipid metabolism and storage. Certain lncRNAs may be involved in the differential expression of genes in visceral versus subcutaneous fat or white versus brown adipose tissues. Dysregulation of these processes can lead to excessive fat accumulation and altered energy balance. Additionally, dysregulated lncRNAs can modulate insulin resistance by affecting adipose tissue function, lipid metabolism, inflammation, and adipokine secretion. Many lncRNAs function by guiding chromatin-modifying complexes to specific genomic loci, thereby influencing the epigenetic landscape of adipose-related genes. This can have long-term effects on fat distribution and metabolism through mechanisms such as the recruitment of chromatin-modifying complexes, interaction with transcription factors, and modulation of non-coding RNAs. Understanding the role of lncRNAs in adipogenesis and fat distribution offers potential therapeutic avenues for managing T2DM. Targeting specific lncRNAs could help improve insulin sensitivity, reduce visceral fat accumulation, and mitigate metabolic dysfunctions associated with diabetes.

7. Role of circular RNAs in Type 2 Diabetes Mellitus pathogenesis

As crucial regulators of gene expression, circRNAs have become a significant focus in RNA biology. The foundation for circRNA research was laid in 2013 with groundbreaking discoveries that underscored their regulatory functions, including their ability to act as miRNA sponging [141, 142]. Although circRNAs were initially studied in various contexts, their relevance to T2DM

caused increased attention to their biological roles and associations with disease states. In 2018, the discovery of *circHIPK3* in T2DM marked a pivotal moment in circRNA research. Stoll and his team conducted a pioneering study revealing that *circHIPK3* is highly expressed in pancreatic β-cells, where it serves as a miRNAs sponge, particularly for mi-124, a key regulator of insulin secretion and β-cells proliferation[143]. Due to its pivotal function and its dysregulation in βeta-cell activity, *circHIPK3* became one of the first circRNAs linked to T2DM. Elevated levels of *circHIPK3* were subsequently identified in the serum of individuals with T2DM, where they were associated with insulin resistance and impaired glucose metabolism. This discovery propelled further research into circRNAs, uncovering additional molecules involved in pathways relevant to T2DM. While the precise role of circRNAs in T2DM remains unclear, growing evidence suggests that these molecules contribute to disease progression through various mechanisms, including effects on insulin resistance, glucose metabolism, inflammation, and lipid metabolism[144].

Performing critical biological functions, circRNAs primarily act as miRNA sponges. By sequestering miRNAs, circRNAs prevent them from interacting with their target mRNAs, thus regulating gene expression. In the context of T2DM, circRNAs influence key pathways, including inflammation, glucose metabolism, lipid metabolism, and insulin resistance. Although research on circRNAs in T2DM is still in its infancy, studying their interactions with miRNAs may provide valuable insights into the molecular mechanisms of the disease and pave the way for novel therapeutic strategies. Plasma circRNAs have emerged as promising candidates for clinical applications due to their association with T2DM pathogenesis and their presence in easily accessible body fluids, such as blood and plasma. These molecules offer the potential for a noninvasive method to monitor disease progression, particularly in high-risk individuals such as those with prediabetes. Since the initial identification of hsa_circRNA_0054633 as a potential biomarker in 2017, circRNAs research has expanded rapidly[145]. Subsequent studies have identified additional circRNAs, including has_circ_0009024[146], has_circ_0071106[147], hsa_circ_0054633 [148], and hsa_circ_0115355[149], which have furthered our understanding of their roles in the pathophysiology of T2DM.

While much of the research on circRNAs has focused on their diagnostic potential, their therapeutic applications in T2DM represent a burgeoning frontier. Although therapeutic circRNAs for T2DM have not been formally discovered or approved, their unique properties, including miRNA sponging, gene regulation, and remarkable stability in bodily fluids, make them appealing candidates for future treatments. Further research is essential to uncover the full potential of circRNAs as diagnostic biomarkers and therapeutic targets in T2DM. Advances in the field could lead to significant breakthroughs in understanding the molecular underpinnings of the disease and developing innovative, noninvasive therapies and interventions.

8. Conclusion

Understanding the link between lncRNAs and T2DM offers numerous advantages, including advancing research, discovering biomarkers, gaining deeper insights into pathogenesis, identifying therapeutic targets, developing predictive models, and enabling personalized medicine. Epigenetic biomarkers have transformed non-invasive molecular diagnostics, surpassing traditional screening and therapeutic techniques. Among these, ccf-lncRNAs hold immense promise in expanding our understanding of complex biological processes, exploring uncharted territories of the epigenomic landscape, and identifying novel targets for therapeutic interventions. As biomarkers, ccf-lncRNAs provide a sophisticated approach to monitoring biological systems in medical diagnostics. These large, conserved molecules act as early warning signals of potential physiological disruptions, making them invaluable for detecting and managing diseases such as T2DM. By enabling earlier and more precise interventions, ccf-lncRNAs pave the way for more effective healthcare strategies. Further research is essential to harness the potential of ccf-lncRNAs. Optimizing their clinical application will require addressing critical aspects, including ethical, legal, and technical. Standardizing protocols for detecting, analyzing, and interpreting is vital for confirming successful integration into clinical settings. As the field advances, ccf-lncRNAs are poised to play a pivotal role in RNA-based therapies and precision medicine. The remarkable properties could revolutionize disease detection and management, allowing conditions to be identified before symptoms arise. This early detection capability could lead to highly personalized treatment potions and significantly improve patient outcomes.

In the future, ccf-lncRNAs integration into clinical practice could transform healthcare by enhancing diagnostic precision, enabling tailored interventions, and fostering a proactive approach to disease management.

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