Chapter 10
Circular RNAs as Potential Biomarkers and Therapeutic Targets for Metabolic Diseases

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Abstract Epidemiological studies provide evidence of a continuous rise in metabolic diseases throughout industrialized countries. Metabolic diseases are commonly associated with different abnormalities that hold a key role in the emergence and progression of frequent disorders including diabetes mellitus (DM), nonalcoholic fatty liver disease (NAFLD), obesity, metabolic syndrome and cardiovascular diseases. The burden of metabolic diseases is believed to arise through complex interaction between genetic and epigenetic factors, lifestyle changes and environmental exposure to triggering stimuli. The diagnosis and treatment of metabolic disorders continue to be an overwhelming challenge. Thus, the development of novel biomarkers may enhance the accuracy of the diagnosis at an early stage of the disease and allow effective intervention. Over the past decade, progress has been made in exploring the potential role of noncoding RNAs (ncRNAs) in the regulation of gene networks involved in metabolic diseases. A growing body of evidence now suggests that aberrant expression of circular RNAs (circRNAs) is relevant to the occurrence and development of metabolic diseases. Accordingly, circRNAs are proposed as predictive biomarkers and potential therapeutic targets for these diseases. As the field of circRNAs is rapidly evolving and knowledge is increasing, the present paper provides current understanding of the regulatory roles of these RNA species mainly in the pathogenesis of DM, NAFLD and obesity. Furthermore, some of the limitations to the promise of circRNAs and perspectives on their future research are discussed.

Keywords Circular RNAs (circRNAs) · Metabolic Diseases · Diabetes · NAFLD · Obesity · Epigenetics · Noncoding RNAs (ncRNAs)

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P. C. Guest (ed.), *Reviews on Biomarker Studies of Metabolic and Metabolism-Related Disorders*, Advances in Experimental Medicine and Biology 1134, https://doi.org/10.1007/978-3-030-12668-1_10

10.1 Introduction

Metabolic diseases refer to different disorders including diabetes mellitus (DM), obesity, metabolic syndrome, dyslipidemia, and non-alcoholic fatty liver disease (NAFLD) [1]. These generally occur when metabolism processes fail. The pathogenesis of metabolic diseases and their chronic complications involve multiple molecular processes and pathways. Early studies using different models revealed that metabolic diseases arise through a complex interplay between genetics, epigenetics, environment, and/or lifestyle factors (nutrition, lack of exercise, etc.) and obesity [2–4]. However, their exact etiology remains partially elucidated.

Despite intensive research into most aspects of metabolic diseases, their causes are still poorly known and only a few effective drugs are available for accurate treatment. Nonetheless, the effectiveness of the current therapy could be improved if it could be implemented at early stage of the disease and targeted to the right subjects who may actually benefit from it. Such an ideal therapy cannot be achieved unless it is combined with predictive biomarkers to guide the treatment. Hence, the search for additional clinically relevant drugs as well as potential biomarkers with precise prognostic and diagnostic value is becoming increasingly important in the field of metabolic diseases.

Recent years have witnessed increasing interest in studying noncoding RNAs (ncRNAs) including long noncoding RNAs (lncRNAs), microRNAs (miRNAs) and circular RNAs (circRNAs), which are considered as important epigenetic regulators of many physiological processes. Huge efforts have been made to use these RNA molecules as predictive biomarkers for several diseases including metabolic disorders [5–9]. Nowadays, the landscape of miRNAs is by far the most characterized in relation to metabolic diseases whereas the role of circRNAs has not yet been precisely defined.

CircRNAs belong to the ever-growing world of ncRNA molecules. They are covalently closed single-stranded molecules generated from precursor mRNA back-splicing [10, 11] and can originate from different genomic regions. The lack of the typical terminal 5′ cap and 3′ polyadenylated tail renders circRNAs more stable and resistant to RNase R digestion compared to the linear RNA counterparts [12–14]. With respect to their biogenesis, detailed mechanisms have not been fully elucidated. Several possible models have been proposed including direct back-splicing with ALU and inverted repeats complementation, exon lariat, and RNA binding protein mediated models [15, 16].

Over the past few years, high throughput technologies have enabled a significant breakthrough in discovery of circRNAs. Today, thousands of circRNAs have been identified and annotated. Based on their genomic location, circRNAs can be classified into at least three types with distinct regulatory functions across multiple mammalian cells and species: (1) exonic circular RNAs (ecircRNAs); (2) circular intronic RNAs (ciRNAs); and (3) exon-intron circular RNAs (EIciRNAs) [11]. EcircRNAs appear to be the most abundant RNAs accounting for over 80% of the already known circRNAs. Moreover, the application of highly sophisticated bioinformatics tools has helped create several circRNA databases with searching and browsing functions [17].

CircRNAs are highly represented in the eukaryotic transcriptome, evolutionary conserved across species, and often show tissue or development stage-specific expression patterns [12, 18–21] suggesting their functional relevance [18, 19, 22, 23]. Interestingly, earlier studies indicated that the expression of a circular RNA does not correlate with the expression of its cognate linear mRNA [24]. In some cases, circRNAs can be more abundantly expressed than their associated linear mRNA isoform [25] while in other situations no circRNA can be detected despite high levels of mRNA expression [22, 23, 26]. The striking expression differences between circRNAs and their mRNA counterparts suggest that the production of circRNAs is a highly orchestrated process [23]. As to their potential functions, research is still limited and challenging. Studies have reported that some circRNAs may act as a sponge for miRNAs via competition with miRNA/mRNA binding or they may interact with RNA-binding proteins (RBPs) or regulate genes at the transcriptional and posttranscriptional levels [11, 27–30]. With these possible functions, specific circRNAs may control essential biological processes and contribute to the pathogenesis of diverse diseases including metabolic disorders [31–33]. However, the exact regulatory mechanisms by which these molecules may carry out these roles are not known. Thus, a more comprehensive understanding of how circRNAs function and what characteristics they should have to interact with other players to orchestrate gene expression in health and diseases states may lay the foundation for the development of RNAs-based diagnostic and therapeutic interventions for complex metabolic diseases. Below I will discuss the most important published studies of circRNAs in DM, NAFLD and obesity. CircRNAs that are most likely to be involved in some of these disorders as well as their putative functions are summarized in Table 10.1.

10.2 CircRNAs and Metabolic Diseases

10.2.1 DM

DM is a multiple-etiology metabolic disorder characterized by chronic hyperglycemia resulting from defects in secretion and/or insulin action [34]. Defects in insulin-mediated uptake of glucose can trigger pathogenic signals including mitochondrial dysfunction, oxidative stress, hypertension, inflammation and dyslipidemia. Additionally, diabetic patients with chronic hyperglycemia are more likely to suffer from many life-limiting and life-threatening complications, such as macrovascular-related stroke, heart disease, peripheral artery disease and/or microvascular-related retinopathy, neuropathy, nephropathy and cancer [35–37]. A major concern with these diabetic complications is that the number of DM cases and associated mortality are constantly increasing globally while the effectiveness of current treatments is limited, and this represents a heavy socioeconomic burden. Thus, identification of novel biomarkers that reflect or predict insulin-secretion dysfunction in individuals could transform the way we deal with diabetes, allowing for early prevention and guided therapy as a step toward precision medicine [38].

Table 10.1 Relevant circular RNAs associated with metabolic diseases

Circular RNA	Expression	Potential function and phenotype	Ref
Diabetes			
CDR1as/cirRS-7	↑	Improves insulin secretion by sponging miR7	[40]
Hsa_circ_0054633	↑	Associated with prediabetes and T2DM in peripheral blood cells Potential biomarker for T2DM	[42]
CircRNA-HIPK3	↑	Promotes retinal vascular disorders by blocking miR-30a-3p function Control of key b-cell functions by sequestering miR-124-3p/miR-138-3p	[44] [41]
CircRNA-0005015	↑	Involved in diabetes retinopathy by acting as miR-519d-3p sponge	[45]
CircANKRD36	↑	Correlated with inflammation in T2DM patient peripheral blood leukocytes Potential biomarker for screening chronic inflammation in T2DM patients	[104]
Hsa-circRNA11783–2	↓	Related to both coronary artery disease and T2DM in peripheral blood	[105]
NAFLD			
CircRNA-0046367	↑	Inhibits hepatic steatosis by preventing hepatotoxicity of lipid peroxidation	[68]
CircRNA-0046366	↑	Inhibits hepatic steatosis through miR-34a/PPARα signaling	[69]
CircScd1	↓	Affects steatosis of NAFLD via JAK2/STAT5 signaling pathways	[71]
Obesity			
CirRS-7	↓	Levels decreased in pancreatic islets of ob/ob and db/db mice	[41]

Ref reference number, *T2DM* type 2 diabetes mellitus

Over the last decade, efforts have been made to understand the disruption of mRNA-miRNA-lncRNA interaction networks under diabetic conditions [39]. More recently, scientists have shifted their research focus to circRNAs, hoping to develop these molecules as new biomarkers for early detection and management of diabetes. In this respect, the most well-known endogenous circRNA related to diabetes in the literature is CDR1as/ciRS-7 (a natural antisense transcript of CDR1) [19, 28]. Overexpression of this circRNA leads to improved insulin production and secretion in mouse β-cells [40]. By acting as a miR-7 sponge [20], CDR1as promotes islet β-cell proliferation and insulin secretion in diabetes via inhibiting miR-7 and enhancing Myrip and Pax6 expression [40]. These encouraging data suggest that the CDR1as/miR-7axis could serve as a potential therapeutic target for the treatment of diabetes. Similarly, another study reported that CDR1as and circHIPK3 silencing in wild-type animal models causes defective insulin secretion and lower islet cell proliferation [41]. By performing microarray and confirming the data by qRT-PCR, Zhao and colleagues measured the differential expression of circRNAs in the peripheral blood of pre-diabetes and T2DM patients compared to matched control

subjects. The most significantly upregulated circRNA was hsa_circ_0054633 (Table 10.1) implying its potential as diagnostic biomarker for prediabetes and type 2 diabetes mellitus (T2DM) in the clinical setting [42]. Circular RNAs have also been investigated in diabetic vascular complications, which are a major cause of mortality among patients with diabetes [43]. In this context, Shan et al. reported that circHIPK3 was significantly induced in the retinas of patients with diabetes [44]. The same study group showed that depletion of circHIPK3 in a mouse model for diabetic retinopathy alleviated the retinal disorder [44]. Mechanistically, circHIPK3 competitively binds different miR-30 isoforms to restore the expression of their target genes including VEGF, FDZ4 and WNT2 which are involved in cell viability, proliferation and migration. In a more recent study, Zhang and colleagues identified circ_0005015 as the most significantly upregulated circRNA in plasma, vitreous samples and fibrovascular membranes of diabetic retinopathy patients [45]. Furthermore, the authors demonstrated that siRNA-mediated silencing of circ_0005015 significantly reduced human retinal vascular endothelial cell proliferation, migration and tube formation. Additional analyses revealed that circ_0005015 acted as an endogenous miR-519d-3p sponge to sequester and inhibit miR-519d-3p, thus facilitating retinal endothelial angiogenic function [45]. Together, these findings suggest that circ_0005015 may be considered as an ideal candidate biomarker for monitoring diabetic retinopathy. CircRNA_000203 is an additional circular transcript linked to diabetes. Tang and colleagues found that circRNA_000203 was upregulated in the diabetic mouse myocardium and in angiotensin (Ang) II-induced mouse cardiac fibroblasts [46]. In fact, circRNA_000203 could specifically increase the expression of fibrosis-associated genes (Col1a2, Col3a1) and α-SMA in cardiac fibroblasts via inhibiting the interaction of miR-26b-5p with the target genes. Therefore, circRNA_000203 might serve as a potential target for prevention and treatment of cardiac fibrosis in diabetic cardiomyopathy [46].

All of the above-mentioned findings suggest that the circRNAs-miRNAs-mRNAs regulatory axis could be a useful therapeutic target for the pathogenesis of diabetes and its complications. However, much more remains to be learned about the biology of circRNAs in diabetes and their beneficial clinical application appears to be a future endeavor.

10.2.2 NAFLD

Non-alcoholic fatty liver disease is emerging as the most common cause of chronic liver disease worldwide. It is a multifaceted disorder that ranges from the simple accumulation of triglycerides in hepatocytes (hepatic steatosis) to steatosis with inflammation, non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis, which may evolve towards cirrhosis and hepatocellular carcinoma [47–50]. The prevalence of NAFLD has been estimated to be between 25% and 45% of the general population [51, 52] and 70–90% among patients with obesity, DM or metabolic syndrome [53–55].

Although the pathophysiology of NAFLD has not been fully elucidated, recent investigations have brought forward evidence that this disorder may be caused by a plethora of factors including hepatic lipid accumulation, adipose tissue and mitochondrial dysfunction, a high fat diet, obesity, a chronic inflammatory state, insulin resistance, and genetic and epigenetic factors [56–58]. NAFLD is clinically important because fatty liver can progress to steatohepatitis in many patients and lead to liver cirrhosis and hepatocellular carcinoma. There is also growing evidence that in patients with NAFLD, hepatic steatosis is closely linked with obesity and the metabolic syndrome [59], which have been well-established as complex metabolic diseases with substantial heterogeneity. It is therefore important to identify biomarkers that may enable earlier prediction and diagnosis of NAFLD and to provide efficient treatment and better management.

An ongoing research effort is attempting to identify biological targets and signals closely associated with NAFLD. Some studies have indicated that miRNAs may have a potential role in this hepatic chronic disease [60]. Indeed, several processes relevant to the development and progression of NAFLD were found to be related to miRNAs [61, 62]. For instance, miR-34 is upregulated in NAFLD and has the potential to be a biomarker for diagnosis of this disorder [63, 64]. Several attempts have been made to translate miRNAs findings to clinical practice. In this sense, Regulus Therapeutics and AstraZeneca have started the development of RG-125, a GalNAc-conjugated anti-miR targeting microRNA-103/107 for the treatment of NASH in pre-diabetes and T2DM patients [65]. Hence, these encouraging findings, combined with the ongoing progress in the field of ncRNAs research, are expected to yield new insights into the pathogenesis of NAFLD.

The circRNA family has also become a key area of focus for research in NAFLD. There is now increasing evidence linking circRNAs to the pathogenesis of NAFLD even though studies in this respect have only just begun. Previous reports have established that the expression of PPARα and associated signaling pathways are inhibited by PPAR1 in patients with NAFLD [66, 67] but the underlying mechanism is not clear. Regarding circRNAs and hepatic steatosis, Guo and colleagues [68] found that circRNA_0046367 was significantly decreased in high-fat-induced hepatic steatosis [68]. Subsequently, the authors demonstrated that the decrease in circRNA_0046367 expression led to miR-34a/PPARα interaction and lipid peroxidative damage, while circRNA_0046367 normalization by intrahepatic overexpression prevented this interaction and therefore reduced steatosis [68]. In a different study, the same authors identified another circRNA, circRNA_0046366, whose expression was also decreased during free fatty acid-induced hepatocellular steatosis and its upregulation abolished the miR-34a-dependent inhibition of PPARα signaling, leading to a marked reduction in triglyceride levels and suppression of hepatocytes steatosis [69]. These findings suggest that the circRNA_0046367/miR-34a/PPARα and circRNA_0046366/miR-34a/PPARα axes play an important role in the pathogenesis of NAFLD [68, 69]. As circRNA_0046367 and circRNA_0046366 have the same target, it would be of interest to examine whether or not these two circRNAs act in synergy and if their transcripts display significant sequence similarities. In another study, the same group used the same model of NAFLD to show

that circRNA_021412 is also associated with hepatic steatosis through the circRNA_021412/miR-1972/LPIN1 signaling cascade [70] (Table 10.1). Finally, in a recent study, Li et al. reported that the expression of circScd1 was significantly lower in NAFLD tissues than control groups whereas its over-expression promoted steatosis of NAFLD via JAK2/STAT5 signaling [71].

Together, the above pioneering studies suggest that circRNAs are potentially involved in NAFLD and have the potential to serve as useful tools for the development of diagnostic and interventional pharmacology. However, as mentioned earlier, circRNA data are still lacking functional evidence and their underlaying mechanisms are still awaiting elucidation. Therefore, further carefully designed prospective studies to emphasize and validate the potential use of circRNAs as NAFLD biomarkers are warranted.

10.2.3 Obesity

Obesity is another chronic metabolic disorder affecting adults and children in developed and developing countries [72]. Genetic predisposition, epigenetics, environment, and lifestyle preferences such as diet and low physical activity play crucial roles in excess body fat development and obesity [73, 74]. Obesity is known to be the main risk factor for several disorders including T2DM, cardiovascular disease, hypertension, coronary heart disease, and certain types of cancers [75, 76]. Due to the considerable impact of obesity on human health, it is therefore essential to develop new strategies with potential for early diagnosis and effective treatment.

While the involvement of miRNAs in the physiological processes of obesity has been closely studied [8, 77, 78], the role of circRNAs remains poorly elucidated. To the best of our knowledge, no groundbreaking studies have ever examined the potential link between circRNAs and obesity in humans. However, examination of the potential impact of circRNAs on diverse metabolic processes and a review of examples in the literature, suggest that circRNAs may play a role in the pathogenesis of obesity. For instance, based on the above studies revealing a significant association between circRNA expression and diabetes and NAFLD, and the fact that both are complications of obesity, it is conceivable that circRNAs may also contribute to the development of obesity. In addition, the antisense non-coding RNA in the INK4 locus (ANRIL), a complex gene with many reported linear and circular isoforms (circANRIL), is generated by the 9p21 locus has polymorphisms that have been associated with increased risk of developing cardiometabolic disease, including type 2 (obesity-related) diabetes and manifestations of atherosclerosis such as coronary artery disease [79–81]. Furthermore, a previous study by Murray et al. reported that lower level of CpG methylation within the promoter of ANRIL at birth is associated with increased cardiovascular risk [82] and adiposity [83] in later childhood. Carrara and colleagues hypothesized in their recent review that ANRIL could be a genomic site of environmental epigenetic influence on obesity [84]. An additional example that would argue in favor of a possible implication of circRNAs

in obesity was shown by Li et al. when they attempted to identify the potential circRNAs associated with adipogenesis and lipid metabolism [85]. The authors analyzed the expression profile of these RNA molecules in subcutaneous adipose tissues of large White pig and Laiwu pig using RNA sequencing technology and bioinformatic methods. Among the differentially expressed circRNAs, they identified circRNA_11897 as the most significantly downregulated while circRNA_26852 was the most significantly upregulated. Subsequent analysis revealed that subcutaneous miR-27a and miR-27b-3p are targets for circRNA_11897 and subcutaneous miR-874 and miR-486 are targets for circRNA_26852 [85]. These target genes are enriched in pathways associated with adipocyte differentiation and lipid metabolism. Since miR-874 and miR-486 were shown to be targets of circRNA_26852, the authors hypothesized that circRNA_26852 may play a role in adipogenic differentiation and lipid metabolism through these miRNAs [85]. On the other hand, since miR-27a is known to promote lipolysis [86] and inhibit adipocyte differentiation by targeting PPARγ [87], it is reasonable to assume that circRNA_11897, which binds miR-27a and miR-27b-3p and consequently provokes upregulation of their target genes, may be implicated in the regulation adipogenic differentiation and lipid metabolism. The fact that several miRNAs have been shown to be involved in the processes of adipogenesis and obesity [8, 88] and lipid metabolism [89, 90], and considering the existing regulatory link and the dynamic interplay between different circRNAs and miRNAs, it is possible to assume that circRNAs may also be part of the complex machinery that orchestrates the regulation of genes associated with obesity. Obesity has been reported to induce a decline in the activity and the amount of PPARγ [91] and an upregulation of miR-130b and miR-138 levels. Considering that miR-130b is known to target 3′-UTR and certain sequences within the coding region of PPARγ [92], while miR-138 indirectly inhibits the expression of PPARγ [93], it is possible that the obesity-associated decline in PPARγ expression may be due to a decline in the expression of yet unknown circRNAs, that normally act as miRNA sponges to target miR-130b. There are reports in the literature that may support this scenario. In a previous study, Deng et al. observed that miR-548 can be regulated by the PPARγ gene, a heart-protective factor shown to be downregulated in acute myocardial infarction (AMI) [94]. Subsequently, when Deng et al. explored the expression profile of circRNAs comparing plasma expression of circRNAs in AMI patients with healthy volunteers, they identified circRNA_081881, which contained seven competitive binding sites for miR-548 as the most significantly downregulated circRNA in AMI. The authors concluded that circRNA_081881 may regulate PPARγ expression by functioning as a competing endogenous RNA (ceRNA) of miR-548 [94].

Collectively, these hypotheses and speculative scenarios are proposed for the purpose of serving as basic framework for further understanding of circRNAs in obesity and providing investigators with potential research directions that may be used for generating new hypotheses for further studies on circRNAs. Finally, as circRNAs research continues, it is expected that new information on the role of these molecules will arise in the field of metabolic diseases. It is hoped that this information will bring evidence for the potential role of circRNAs in metabolic diseases.

10.3 Conclusions and Future Perspectives

Even though circRNAs are increasingly being recognized to play critical regulatory roles in the development of metabolic diseases, the lack of their large exploration and characterization may delay their consideration for clinical settings. In this respect, many concerns are left for their potential future studies. The analytical approaches used in the identification and prediction of circRNAs are still part of a relatively new field of investigation, thus, their sensitivity and specificity require improvement. Furthermore, lack of prospective studies, poor study design and complicated statistical analyses could impede the translation of circRNA results to preclinical and clinical trials, thus, limiting the success of prospective biomarkers. The complex interplay of circRNAs with networks involving transcription factors, mRNA, miRNAs, RBPs and metabolic pathways makes it difficult to evaluate the functions of these RNA molecules under complex metabolic diseases. It should also be noted that circRNAs as transcriptional and posttranscriptional regulators themselves undergo extensive regulation from their biogenesis to the effects that they exert on their target molecules and pathways. Therefore, interpretation of such complex data could be enhanced by deploying systems biology approaches to refine our understanding of circRNAs dynamic and provide insights into their potential regulatory circuits in metabolic disorders. Another limitation that may generate huge incoherencies in circRNA results within a group of patients with metabolic diseases is drug use and other treatment modalities not taken in consideration. Using the example of miRNAs, previous studies reported that statins [95, 96], anticoagulation [97], and antiplatelet drugs [98] can affect quantification of these RNAs in blood samples and therefore should be taken in account. Regarding the patients with metabolic disease, thiazolidinedione drugs are frequently used for patients with impaired fasting glucose tolerance while abdominal obesity can be treated with a variety of lower calorie diets along with regular exercise [99]. Hence, drugs as well as confounding parameters should be also taken in account when examining circRNAs in patients with diabetes, NAFLD, obesity, and metabolic syndrome, as these may impact the disease through these RNA species. Metabolic diseases represent a cluster of disorders such as T2DM, insulin resistance, metabolic syndrome, NAFLD and hypercholesterolemia, which could be linked by numerous metabolic pathways. The interplay between these clinical situations is challenging. Thus, although each of these disorders has different physiological and clinical symptoms, it would be important to identify a signature or set of markers including circRNAs, shared by all disorders constituting metabolic diseases. This idea proses that, rather than relying on a single circRNA biomarker for disease diagnosis, one can use a group of disease-relevant biomarkers which will likely be more accurate and efficient in predicting a complex phenotype. Another type of circRNA that has not been well explored in metabolic diseases is circRNA found within exosomes (exo-circRNAs). The presence of abundant circRNAs within exosomes was firstly reported by Li and colleagues [100] and a web-accessible database (http://www.exoRBase.org), exoRBase, a resource containing all available long RNAs (circRNA, lncRNA and mRNA) derived from RNA-seq data of human blood exosomes, has been recently constructed [101].

With respect to metabolic diseases, many questions remain uncertain. For instance, what is the role of exo-circRNAs in metabolic disorders? What is their origin? Are they horizontally transferred via exosome vehicles to recipient cells as in the case of mRNAs [102]? In studies of cancer, Li et al. [100] observed that the abundance of tumor-derived exo-circRNAs in the serum of patients with colorectal cancer was correlated with tumor mass. They also found that the expression profile of exo-circRNAs in cancer serum was significantly different from that in normal serum. More importantly, a recent study revealed that treatment of lean mice with exosomes isolated from obese mice induced glucose intolerance and insulin resistance in mice [103]. Hence, future studies should aim for answering these questions in order to understand the origin, mode of secretion, target cells and organs of exo-circRNAs. This knowledge may help us to gain more insights into the function of circRNAs in the field of metabolic diseases. With respect to the epigenetic regulation of complex metabolic diseases by ncRNAs, there are only a few published data associating the dysregulation of circRNAs with genes involved diabetes and NAFLD, as mentioned above. Unfortunately, no data are yet available on the potential implication of circRNAs in obesity and metabolic syndrome. Likewise, no reports are available on the potential link between circRNAs and the chronic low-grade inflammation associated with diabetes, obesity and the metabolic syndrome apart from one report indicating an association between circANKRD36 and inflammation in patients with T2DM [104]. All of these pertinent questions represent important issues that must be solved in future investigative attempts to fully understand the role of circRNAs in the pathogenesis of metabolic diseases.

In summary, although the existing studies support a possible association between circRNA molecules and metabolic diseases, it is too early to consider and develop these molecules as sensors and biomarkers for metabolic disorders as claimed by existing reports. Further research in this area is worthwhile and new powerful strategies should be employed to uncover the full biological relevance of circRNAs and their potential therapeutic applications.

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