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Chapter 13

Micro-ribonucleic Acids in Diabetes: Robust Biomarkers of Prevention and Precision Medicine

Muthuswamy Balasubramanyam, Paramasivam Prabu, Chandrakumar Sathishkumar, Aravind Sankaramoorthy, Viswanathan Mohan

INTRODUCTION

Diabetes is undoubtedly one of the most challenging health problems of the 21st century worldwide. According to the report from the International Diabetes Federation diabetes atlas 7th edition, the global prevalence of diabetes is estimated about 415 million, with India alone harboring a huge burden of more than 69 million diabetics.[1] In a real-time survey, Indian Council of Medical Research-India Diabetes study, the first national-state-wise representative survey of diabetes in India has also reported an outrageous 62.4 million people with diabetes and 77.2 million people with prediabetes.[2] Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder characterized by hyperglycemia resulting from β-cell failure (impaired insulin secretion) and insulin resistance (defect in insulin action). It is evidenced that complex interplay among genetic, epigenetic, and environmental factors ultimately steering the disease initiation, progression and development of diabetes, and its complications. Identification of strong genetic factors has been a challenging task in the field of diabetes. The recent technological developments including Genome-Wide Association Studies and next-generation sequencing have explored more than 120 gene variants that showed association with type 2 diabetes and many more with diabetes related traits. However, these genetic variants only explain small proportion of the total risk for the T2DM and remaining more than 75% risk are in the dark phase of the research that calls for epigenetic studies. Understanding the mechanisms of epigenomics in relation to the pathogenesis of diabetes etiology may work for prevention aspects as well as for treatment and avoiding diabetic complications. Thus epigenetic modification [deoxyribonucleic acid (DNA) methylation, histone modifications, and micro-ribonucleic acid (miRNA) alterations] are now increasingly received attention in the context of insulin resistance and β-cell dysfunction. Because of their defined functions as key post-transcriptional regulatory mediators, miRNAs are rapidly emerging as key players in the pathogenesis of a variety of diseases including type 2 diabetes. In this review, miRNAs are discussed as biomarkers for metabolic diseases with special reference to type 2 diabetes and a focus on circulatory miRNAs. The authors summarized some of the clinical evidence for the use of miRNAs as biomarkers in T2DM, their utility in dietary and lifestyle modification avenues, and some of the challenges and road-map milestones in future miRNA diagnostics and personalized or precision medicine.

SIGNIFICANCE OF EPIGENETICS AND MICRO-RIBONECLEIC ACIDS

International human genome project identified approximately 25,000 genes; these genes need the instruction for what to do, when to do and where to do and that's where the epigenome comes into the play.[3] Epigenome is nothing but the addition of the second genome to already available genome. As the living organism undergoes growth and development, maneuvering chemical reactions activate and deactivate parts of the genome at strategic times and in specific locations. Epigenetic information are stored as the covalent chemical modification to cytosine bases of the DNA, histone/nonhistone protein or RNA, resulting in changes to the function and/or dynamic regulation

of these molecules without altering the primary DNA sequences of the genome. By systemic regulation of the chromatin structure and DNA accessibility, these chemical changes influence how the genome is made programmed and expressed during the developmental stages like organ development, tissue type, and diseased state.[4] In some cases, epigenetic modifications are stable and passed on to future generations, but in other instances they are dynamic and change in response to environmental stimuli. Nearly every aspect of biology is influenced by epigenetics, making it one of the most important fields in science. Aberrant epigenetic modifications are well recognized drivers for the disease phenotype. Epigenetic modifications include DNA methylation, histone modifications, and noncoding RNA (ncRNAs) regulation.

One of the several regulatory check points in gene expression in higher eukaryotes identified recently is the regulation of transcription and translation by miRNAs. MicroRNAs represent a class of small ncRNAs with specific functions in control of gene expression at post-transcriptional level.[5] The miRNAs mature form is a single stranded RNA, 19-22 nucleotides long, whose maturation steps take place in part in the nucleus and in part in the cytoplasm. Since miRNAs are involved in regulation of a wide variety of processes (including the whole body metabolism), the regulation of miRNAs by itself forms a critical component in the proper regulation of important cellular processes and dysregulation of miRNA expression will ultimately manifest as pathological disease-states including type 2 diabetes.

WHAT MAKES MICRO-RIBONUCLEIC ACIDS AS ROBUST BIOMARKERS?

According to the National Institutes of Health Biomarkers Definitions Working Group,[6] an ideal biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." Although biomarkers come in many forms including DNA mutations, proteins, and messenger RNA (mRNA) transcripts, ncRNAs have become the focus of recent biomarker research because of their unique characteristics. Among several types of ncRNAs, miRNAs are considered as ideal biomarkers as they qualify and fulfill all the stringent features and characteristics. In fact, clinical applications of miRNAs for several diseases including diabetes are under much hype because of the robust biomarker characteristics of miRNAs (Table 1). This includes noninvasive sampling, miRNA stability, tissue specificity, representative physiology, and discriminative power with regard to disease state and increased prognosis/diagnosis accuracy.

TABLE 1: Unique Biomarker Characteristics of miRNAs

Biomarker Characteristics	Salient Features	Potential advantage
Non-invasive biomarkers	miRNAs are secreted into circulation and can be measured in all biological fluids. miRNAs detected in a variety of body fluids, including plasma, serum, urine, tear, vitreous, saliva, semen and breast milk.	Clinical application
Remarkable stability	miRNA has been reported to be remarkably stable in human plasma and serum in spite of high endogenous RNase activity in the circulation. Besides RNase activity, the serum miRNAs were resistant against treatment with harsh conditions, including digestion with RNase or DNase, low/high pH, extended storage or freeze-thaw cycles	Potential clinical utility and translational application
	miRNAs can be isolated and evaluated from formalin-fixed paraffin-embedded (FFPE) samples	Potential for retrospective studies on a large number of samples
Tissue specificity	miRNA expression profiles are specific to tissue origin	Distinguish underlying pathology of disease genesis and progression
	Exosomal miRNA might reflect tissue of origin	'Remote diagnosis' of tissue pathology - Replacement of biopsy - Liquid biopsy utility
Representative of physiology	miRNAs have been associated with specific physiological and pathological states	Decreased complexity compared with mRNA and/or protein expression

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Biomarker Characteristics	Salient Features	Potential advantage
	Many physiological states are under the control of few miRNAs	A simple panel of miRNAs translate a status of health and disease
Discriminatory power	miRNA-based classifiers perform better than mRNA-based classifiers in identifying the subclinical status of the disease states	Increased prognostic/diagnostic accuracy Distinguish between active disease and early organ damage

DIFFERENTIALLY EXPRESSED TISSUE-SPECIFIC MICRO-RIBONUCLEIC ACIDS LINKED TO TYPE 2 DIABETES MELLITUS

MicroRNAs regulate a wide variety of processes of β-cell related insulin secretion as well as insulin target tissues such as skeletal muscle, liver, and adipocytes which are majorly involved in the pathogenesis of type 2 diabetes. Literature related to this area is very exhaustive and several reviews have been published recently.[7-9] A direct evidence of the role of miRNA in diabetes came from the work on pancreatic β-cells where miR-375 and miR-376 are involved in the glucose stimulated insulin secretion by controlling the expression of myptrophin which controls the binding of insulin containing vesicles to the plasma membrane.[10] A detailed review on the role of miRNAs in the control of β-cell activities has emphasized miRNAs as key regulators of β-cell physiology and insulin secretion cascade.[11] Unfortunately, many of the studies investigating the role of specific miRNAs in β-cells were carried out only in cell lines and animal models, and there is an imperative need for more clinically relevant studies. One of the milestone human skeletal studies has evidenced differentially expressed miRNAs in subjects with impaired glucose tolerance (IGT) and patients with T2DM compared to individuals with normal glucose tolerance. In this study, about 30% of the expressed miRNAs are dysregulated in skeletal muscle from people with T2DM, suggesting an important role for miRNAs in the development of skeletal muscle insulin resistance.[12] Interestingly, this was seen despite no significant changes in the global mRNA transcriptome. Differential expression of miRNAs in skeletal muscle was also reported from studies of twins with and without T2DM.[13] Certain myomiRs (miR-1, miR-133a/b, and miR-206), which are important for muscle development and differentiation were shown down regulated in skeletal muscle from patients with T2DM compared to control individuals.[12]

DIFFERENTIALLY EXPRESSED CIRCULATORY MIRO-RIBONUCLEIC ACIDS LINKED TO TYPE 2 DIABETES MELLITUS

Starting from the year 2010 to till date, several studies in different ethnic populations have reported the biomarker role of differentially expressed circulatory miRNAs linked to T2DM (Table 2). One of the pioneering studies by Zampetaki et al.[14] reported a panel of five miRNAs (miR-15a, miR-126, miR-223, miR-320, and miR-28-3p) that were able to distinguish T2DM patients from healthy controls. They have showed specific loss of endothelial miR-126 in T2DM patients implying a loss of angiogenic potential which is linked to cardiovascular risk. In one of our pilot studies, the authors have also shown downregulated expression of miR-126 in plasma from patients with T2DM[15] as the same was reported in another ethnic study.[16] Using serum/plasma/peripheral blood mononuclear cells samples (PBMCs), several studies have shown differentially expressed miRNAs in patients with T2DM.[17-23] Karolina et al.[24] have also observed dysregulation of seven candidate miRNAs in individuals with metabolic syndrome. Despite the increasing attention worldwide on miRNA studies in relation to diabetes, there is limited data on Indians. Although the link between inflammation and insulin resistance/T2DM is well known, there is an imperative need to study the upstream cellular mechanisms that regulate the complex machinery of inflammatory network. In this context, the authors have shown impaired miR-146a levels in PBMCs from T2DM patients with accompanying augmentation of proinflammatory target genes such as tumor necrosis factor (TNF)-receptor associated factor-6 and NFκB and circulatory levels of TNF-α and interleukin (IL)-6.[25] Interestingly, miR-146a levels exhibited negative association with insulin resistance and glycosylated hemoglobin and appears to be a potential biomarker. Recently, one of our milestone

TABLE 2: Circulating micro-ribonucleic acid differentially expressed in samples from patients with type 2 diabetes mellitus

Sample type	miRNA status in samples from T2DM		Impact	Reference Nos.
	Upregulated	Downregulated		
Plasma	miR-28-3p	miR-15a, miR-29b, miR-126, miR-223	miRNA signatures for T2DM revealed with specific loss of endothelial miR-126	14
PBMCs	–	miR-146a	Impairment of miR-146a linked to inflammation and insulin resistance in T2DM	25
Serum	miR-9, miR-29a, miR- 30d, miR-34a, miR-124a, miR-146a, and miR-375	–	Seven miRNAs shown upregulated in serum from newly diagnosed T2DM patients	17
Blood	miR-150, miR-192, miR-29a, miR-320a, miR-144	miR-146, miR182, and miR-30d	Identification of possible predictors of miRNAs for newly onset T2DM	18
PBMCs	–	miR-126, miR-21, miR-27a, miR-27b, and miR-130a	miR-126 is downregulated in endothelial progenitor cells from diabetic patients	19
Blood	miR-27a, miR-320a	miR-197,miR-23a, miR-509a-5p, miR-130b, and miR-195	Dysregulation of seven candidate miRNAs in individuals with metabolic syndrome	24
Plasma	–	miR-126-3p	Decreased expression of miR-126 in T2DM as a biomarker	15, 16
PBMCs	miR-144, miR-150, miR-192, miR-29a, and miR-320	miR-146, miR-182, and miR-30d	Link of circulating microRNAs with intracellular signaling pathway in IGT/T2DM patients	20
Serum	miR-15b	miR-138, and miR-376a	Potential miRNA biomarkers for T2DM/obesity	21
Plasma	miR-140-5p, miR-142-3p, and miR-222	miR-423-5p, miR-125b, miR-192, miR-195, miR-130b, miR-532-5p, and miR-126	Dysregulation of miRNAs in patients with T2DM	22
Serum	miR-128, miR-130b-3p, miR-374a-5p	miR-423-5p	Circulating miRNAs of "Asian Indian Phenotype" identified in subjects with IGT and T2DM patients	26
Serum	miR-320d, miR-4534, miR- 3960, miR-451a, and miR-572 (differentially expressed in T2DM)		Potential serum biomarker of miRNAs in T2DM	23

miRNA, micro-ribonucleic acid; T2DM, type 2 diabetes mellitus; PBMCs, peripheral blood mononuclear cells samples; IGT, impaired glucose tolerance.

studies[26] revealed circulatory miRNAs of "Asian Indian Phenotype" as evident from the 4 differentially expressed miRNAs (miR-128, miR-130b-3p, miR-374a-5p, miR-423-5p) in subjects with IGT and T2DM patients compared to control subjects. Among the altered circulating miRNAs, miR-128 had never been described in previous studies/populations and appeared to be a "new lead" in Indians.

Several studies also imply a promising biomarker role of miRNAs in the genesis of diabetic retinopathy and diabetic nephropathy (DN). A panel of five miRNAs (miR-571, miR-661, miR-770-5p, miR-892b, and miR-1303) was shown elevated in serum from T2DM patients with multiple microvascular complications including diabetic retinopathy, diabetic neuropathy, DN, and diabetic foot.[27] Using urinary exosomes, Delić et al.[28] have identified 16 miRNAs that were differentially regulated in microalbuminuric T2DM patients with DN. Findings from Chien et al.[29] also showed that miR-21, miR-29a/b/c, and miR-192 could reflect diabetic nephropathy pathogenesis and serve as biomarkers during DN progression. Further prospective and larger cohort studies are needed to determine the prognostic/diagnostic value of circulating miRNAs in diabetes and its complications.

MICRO-RIBONUCLEIC ACIDS REGULATION BY NUTRIENTS

Recent studies emphasize that nutrition and dietary components as significant epigenetic factors to have an important role in posttranscriptional regulations of lipid and glucose metabolism genes by modulating the related key miRNAs.[30,31] Several studies demonstrate that

dietary components including macronutrients (proteins and amino acids, carbohydrates, and fatty acids) and micronutrients (vitamins and minerals) could exert some of their epigenetic effects through influencing miRNAs expression and functions. In this context, several polyphenols (resveratrol, proanthocyandin, curcumin, green tea catechins) are now assuming new roles as epigenetic/post-transcriptional modulators of metabolic pathways including lipid and glucose metabolism. As studies are unraveling the role of absorption of food derived exogenous miRNAs[32] and breast milk miRNAs with immune-modulator activities[33] in humans, much more new-biology insights are expected in the field of miRNA regulation by nutrients. Therefore, in future, miRNA profiling can be a useful aid for designing therapeutic approaches, assessment of the nutritional status and planning suitable diet (personalized nutrition) in the prevention as well as control of metabolic diseases including T2DM.

MICRO-RIBONUCLEIC ACID AND PHYSICAL ACTIVITY/PHYSICAL INACTIVITY

Recent studies demonstrated that miRNAs were also involved in the regulations of biological processes induced by physical activity. Both, acute and chronic exercise protocols have been shown to modify the levels of miRNAs in blood and in a variety of tissues.[34-36] It has been shown that miRNA expression can be regulated differently in training "responders" and "nonresponders".[34] As blood of individuals tested 24 hour after the marathon run reveal miR-1, -133a, and -206 correlated well with oxygen uptake (VO_{2max}) and running speed, these miRNAs are potentially considered as biomarker of aerobic capacity.[37] Very recently, Gastebois et al.[38] showed that decreasing physical activity lead to an increase in miR-148b muscle content in human and mice with mechanistic signaling actions in relation to insulin sensitivity and glucose metabolism. These studies emphasize specific miRNA signatures linked to physical inactivity and exercise responses which should be exploited by further studies towards appropriate prevention strategies for metabolic diseases like T2DM and associated disorders.

POINT-OF-CARE BASED MICO-RIBONUCLEIC ACIDS DIAGNOSTICS

Developing a portable, easy to use miRNA detector with sufficient selectivity and sensitivity for point-of-care diagnostics is the need of the day. Among different approaches that have developed for circulating miRNA detection, biosensors, due to the high sensitivity, ease of use, short assay time, nontoxic experimental steps, and adaptability to point-of-care testing, exhibit very attractive properties for developing portable device.[39,40] Synthetic organic and polymer chemistry approaches are also used to prepare miRNA sensors as components of a hand-held, easy-to-use, sensitive device that can be used to assist with patient screening, early diagnosis, and disease monitoring. A power-free microfluidics chip with laminar flow-assisted dendritic amplification is being under testing for detection of miRNAs in clinical samples.[41] A "digital microfluidic" platform is also under development and testing for the detection of miRNAs from blood with an aim to make the technology available to labs and healthcare providers in limited resource settings. The platform encompasses (i) a molecular assay capable of semiquantitative reporting of miRNAs in biofluids, (ii) an instrument that fully automates the assay and minimizes user effort to simple deposition sample in a digital microfluidics cartridge inlet, and (iii) real-time data analysis through cloud computing.

CONCLUSION

MicroRNAs have come a long way since the initial discoveries of more than two decades ago. Their emerging potential as biomarkers in clinical diagnostics as well as modulators for the treatment of a variety of diseases including diabetes is truly exciting. In the near future, it will become clearer as to whether they have the power to become established new molecular diagnostic benchmarks and whether microRNA-based diagnosis and therapy can compete with that of selective protein inhibitors. Prior to the use of miRNAs for diagnostic purposes, much more needs to be standardized with regard to the methods of miRNAs detection in well annotated samples and adequately quality controlled data processing. Before translation into clinical practice, all circulating miRNA findings require further steps of validation and an accurate standardization of all preanalytical and analytical procedures so as to pave for the development of a universal platform of noninvasive, reliable miRNA detection tool of precision medicine quality. Collective and coordinated efforts by clinicians, academicians, and industrial researchers are needed to position and promote the transfer of miRNAs diagnostics from bench to bedside.

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