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REVIEW ARTICLE

Blood miRNA signature in Type-2 Diabetes Mellitus: Possible futuristic molecular biomarkers.

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ABSTRACT… The disease burden associated with Type-2 Diabetes Mellitus (T2DM) increasing on a daily basis, which makes it necessary to develop new diagnostic modalities for T2DM. The recently emerging molecular biomarkers have high predictive potential, enhanced diagnostic efficacy, therapeutic efficacy, and ability to assess progression to complications. In recent times we have some epigenetic biomarkers like miRNAs which have shown promise in various diseases including T2DM. There are recent reports that some epigenetic biomarkers, including miRNA have the predicting the onset of various diseases including T2DM. Recent data highlights the diagnostic potential of miRNAs in the diagnosis, associated pharmacogenomics, and assessment of various microvascular and macrovascular complications in T2DM. The existing literature was reviewed as per a defined methodology, and the studies on the miRNAs in the blood samples of patients with diabetes were finally included herein. The review identified the miRNAs that are upregulated and downregulated across various stages of T2DM. A T2DM miRNA signature was finally identified, which needs to be further validated by replicative data obtained from high-quality trials or advanced sequencing techniques for confirmation or annulment.

Key words: DGCR8, miRNA, Messenger RNA (mRNA), pri-miRNA, pre-miRNA, Type-2 Diabetes Mellitus (T2DM).

INTRODUCTION

Ever since the discovery of the "double helix structure" of DNA by Watson and Crick for which they won the Noble Prize, scientists have taken almost 65 years to explore the function of various micro mechanics and nano tools for deciphering the translation of the genetic code into proteins that support "life" on this planet. One of the novel discoveries in molecular pathology was the discovery of miRNA (micro RNA) which regulate protein synthesis, as biological tools.[1] Numerous mediators, including chemicals or ligands and their receptors partake in signaling pathways that are governed and regulated by various regulatory elements, including miRNAs.[2] miRNAs are very small 22 nucleotides (nt) long non-coding RNAs that act as a regulatory structure and induce mRNA silencing by destabilizing or inhibiting mRNAs via different mechanisms, which in turn destabilizes the newly formed DNA.[3] A single miRNA may cleave multiple mRNA targets and vice versa.[4] The details of miRNA genesis to miRNA induced cleavage are depicted in figure-1. The physiological function of miRNA is to regulate the various the signaling pathways in molecular regulation of multi-faceted actions that lead to shaping up of proteins for various physiological actions.[4] The degradation of messenger RNAs (mRNA) is a programmed cellular phenomenon, and malfunctions in miRNA degradation can lead to various metabolic alterations.

Various metabolic disorders including obesity, cancers and insulin resistance syndrome are presently surfacing as the major killers on our planet, and are the most prevalent human diseases as revealed by world morbidity and mortality. Recent data have revealed that the disease-associated mortality and morbidity of diabetes mellitus, and specifically type-2 diabetes mellitus (T2DM), are immense.[5] To date, conventional medical knowledge relies on biomarkers that

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have been identified over the last century or so, including different modes of glucose-based analytics, levels of glycated hemoglobin, and glycated albumin levels. Although these methods have been optimized over time and the accuracy and precision of these methods have improved following validation using large volumes of data, recent literature demonstrate that these methods have certain shortcomings that are obvious when the heterogeneous nature of T2DM is considered.[6] This notion is further supported by the evolving concept of predictive medicine in preempting diseases and new tailor-made evidence for the clinical market. With the increasing practice of gene therapy, the potential targets, genetics, and molecular biomarkers will probably become more valuable in documenting the actual therapeutic success of the strategy.[7] The use of molecular tools seems inevitable with the well-established need for more efficient and predictive biomarkers for the diagnosis of diabetes, as they should be able to provide information regarding the pharmacogenomics, classify the prognosis of diabetic patients, and identify familial patterns. Therefore, a novel molecular biomarker is urgently necessary for meeting the requirements of molecular medicine.

While genetic markers are already being studied, certain epigenetic biomarkers, including methylated gene sequences and miRNAs have emerged as molecular tools for obtaining deeper insights into diabetes mellitus.[8] The aberrant expression of miRNAs has been associated with several pathological disorders. Conventional medicine needs to be upgraded and replaced by molecular medicine in areas of predictive disease modeling and onward gene therapy. The recent emergence of miRNA biomarkers can help meet the shortcomings for targeting perfection, better predictive efficacy, and the precise identification of genetic defects.[9]

It therefore follows that it is necessary to identify a biomarker that will be able to predict, diagnose, monitor, and maesure the prognosis of T2DM. With this background, a systematic review was conducted, which summarizes the present knowledge on the expression levels of miRNAs in human subjects with diabetes.

miRNAs-

miRNAs are very small nt, 22 long intronic RNAs that essentially regulate post-transcriptional gene functions by modifying the translation of proteins from messenger RNAs (mRNAs). miRNAs usually originate from the non-coding sequences of genes, that can be easily edited to IsomiRs, with coding sequences slightly different from that of the miRNAs. The miRNA precursors are known as pri-miRNAs, which contain a stem-loop structure at one end, two overhangs at the capped 5′-end, and a polyadenylated cap at the 3′-end. The pri-miRNA faces within the nucleus a microprocessor complex containing DGCR8 and Drosha, which cleaves the two overhangs to form a pre-miRNA. The pre-miRNA is transported from the nucleus through the exportin-5 channels into the cytoplasm, where the pre-miRNA is cleaved by the DICER protein, resulting in the formation of 22 nt long miRNA duplexes. Subsequently, one of the strands of this duplex becomes inactivated, while the other strand, known as the guide strand, is incorporated into the RNA-induced silencing complex (RISC), to finally degrade the mRNA. The inactivation of the mRNA by the miRNA is referred to as “RNA silencing”.[10] The biogenesis and subsequent mRNA silencing is briefly described in Figure-1.

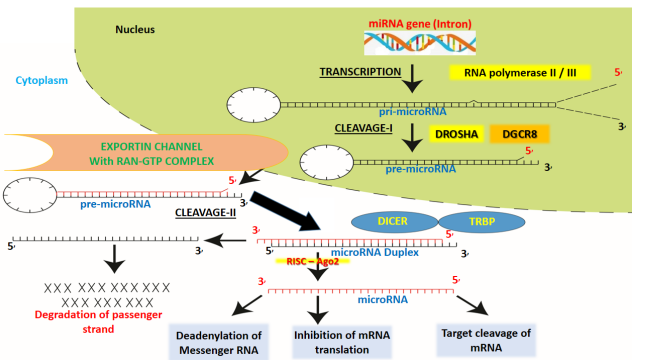

Figure-1. Schematic representing miRNA biogenesis, cleavage and inhibition of mRNA.

Step- I: pri-miRNA transcription from intronic region of gene, **Step-II:** pri-miRNA cleavage at 5′ and 3′ overhangs by Drosha and DGCR8 to form pre-miRNA, **Step-III:** Movement of pre-miRNA from nucleus to cytoplasm through Exportin-5 channel, **Step-IV:** Further cleavage of pre-

miRNA to form miRNA duplex by DICER protein, **Step-V:** Degradation of passenger strand and incorporation of guide strand into RISC complex to inhibit mRNA function.

Owing to their mRNA silencing function, miRNAs have enormous potential, that can be used to silence various proteins and subsequently inhibit the function of multiple metabolic pathways. The pre-translational role of miRNAs in regulating the various key enzymes of glycolysis and gluconeogenesis is well-studied.[11] The role of miRNAs in adipose tissue metabolism, which can modify the glucose sensitivity of various signaling pathways which have been studied by several authors.[12] miRNAs are found in nearly all the tissues and body secretions, which explains the current shift in diagnostic tools, from histopathological specimens to blood-based methodologies. Blood serum, and plasma-based detection methods are emerging as biomarkers for the detection of various diseases, and have immense potential for future diagnostic studies.[13]

MATERIAL & METHODS

The PubMed database was searched using the keywords "blood microRNA in diagnosis of diabetes mellitus", and retrieved a total of 265 studies. The filter "human" was applied, and the number of search items was reduced to 217 articles. The search results were further confined to articles that had been published in the last 5 years, which returned 158 articles. Then, only those articles with free full text (n=79) were selected. Further studies and research led to more exclusions, including 10 studies that included pregnant subjects, patients with type-1 diabetes mellitus (n=8), studies not directly evaluating the miRNA in blood (n=14), studies that evaluated circular RNA (n=3), and studies that did not directly study T2DM or T2DM-related complications (n=14) between January 2019 and January 2020. A total of 33 studies were finally shortlisted, which were studied in further detail. The key outcome measures were documented, including the upregulation or downregulation of miRNAs in T2DM.

RESULTS

The data review highlighted the fact that multiple miRNAs were expressed or downregulated during the course of T2DM. Although the cause and effect of several cases are poorly understood, it is essential for diagnostic modalities to explore the potential of various miRNAs in disease prediction and segregating early disease that is, segregating pre-diabetes or impaired glucose tolerance from advanced T2DM. These markers can also help identify underlying T2DM associated with microvascular and macrovascular complications. Owing to these features, the emerging role of miRNAs is thought to have numerous applications, including monitoring the treatment response to a particular T2DM therapy. Although several studies did not report consistent changes in miRNA expression, some data revealed significant upregulation or downregulation of miRNA expression in subjects with T2DM. The miRNAs that were reported to be upregulated or downregulated in patients with T2DM, the strategy employed for identifying a particular disease stage, and the complications of T2DM are enlisted in Table-I and Table-II.

The signs of epigenetic triggers, namely the changes in miRNA expression, can possibly result from other types of pathological conditions, including infections, autoimmune disorders, and related metabolic diseases. Some authors have identified certain miRNAs as markers of acute phase reactions, such as sepsis, where the expression of a set of miRNAs may be deregulated.[45] Author is of the opinion that the deranged expression pattern of miRNAs that arise from an inflammatory phase reaction can be attributed to acute metabolic or non-metabolic insult that can disturb the pattern of presentation of miRNAs for other diseases. However, any disease process is associated with a certain degree of inflammation, which is accompanied by specific changes in the organs or the pathology. Therefore, the alterations in the expression patterns of the miRNAs described above hint towards a possible "miRNA T2DM signature" which can be studied in further details for clinical applications.

Up-regulated miRNA	Salient features of the study	Ref
miR-99b, miR-122-5p	Associated with pre-diabetes and newly diagnosed diabetes	Regmi et al[14]
miR-21	Levels of miR-92a were raised in diabetic subjects with Acute Coronary Syndrome (ACS), in comparison to non-diabetic ACS	La Sala et al.[9]
miR-92a	Levels were raised in subjects with diabetes before onset	Wang et al[15]
miR-150 miR-30a-5p	Associated with pre-diabetes and newly diagnosed diabetes	Jiménez-Lucena et al[16]
miR-4739	Levels were upregulated only in T2DM with critical limb ischemia (CLI), in comparison to that of patients without limb ischemia	Li et al[17]
miR-483-5p	The expression of miR-483-5p was linked to the incidence of diabetes and insulin resistance	Gallo et al[18]
miR-323b-5p	Levels raised in diabetics with CLI in comparison to those of diabetic patients without CLI	Cheng et al[19]
miR-126, miR-770	Upregulated in patients with T2DM and nephropathy	Park et al.[20]
miR-19a miR-126	Levels found to correlate with pro-thrombotic factors in diabetes mellitus	Witkowski et al.[21]
miR-122 miR-99	Non-progressive subjects with pre-diabetes had greater changes in miRNA expression than progressive pre-diabetics	de Candia et al[22]
miR-486, miR-146b miR-15b	Could predict the development of T2DM in obese children	Cui et al.[23]
miR-196a2 miR-1908	Associated with the regulation of fat metabolism and glycemic parameters	Ghanbari et al.[24]
miR-9 miR-370	Levels raised in patients with T2DM and patients with T2DM and coronary artery disease	Motawae et al.[25]
miR-155	Raised in proliferative and background diabetic retinopathy in comparison to non-diabetic retinopathy	Yang et al.[26]
miR-130a(Plasma), miR-802 (Serum), miR-26(Exosomes)	Blood levels raised in insulin resistance	Deiuliis.[27]
miR-21,miR-29a/b/c,miR-192	Associated with the regulation of fat metabolism and glycemic parameters	Chien et al.[28]
miR-375, miR-21, miR-24.1, miR-30d, miR-34a, miR-126, miR-146, miR-148a	Levels raised in patients with T2DM and patients with T2DM and coronary artery disease	Seyhan et al.[29]
miR-33b	miRNA33 with correction for miR-16 were up-regulated in T2DM	Kimura et al.[30]
miR-21	Increased in T2DM with background and proliferative retinopathy in comparison to patients without retinopathy	Jiang et al.[31]
miR-29a-3p, miR-122-5p, miR-124-3p, miR-320a	The upregulated miRNAs were downregulated following bariatric surgery	Zhu et al.[32]
miR-27b	NGS and qRT-PCRdid not show consistent findings for most miRNAs among diabetic and normoglycemic subjects. Only miR-27b was upregulated in diabetic individuals.	Dias et al.[33]
miR-223-3p	Can serve as a potential biomarker for diabetic nephropathy	Zhang et al.[34]

Table-I. Up-regulated miRNAs in last 5 year data review from literature

Down-regulated miRNA	Study Salient	Reference
miR-3666	The expression of miR-3666 was negatively related to diabetes, adiponectin and insulin resistance	Tan et al.[35]
mir-20a miR-486	The expression of these miRNAs was downregulated, especially in patients with diabetic kidney disease	Regmi et al.[36]
miR-15a miR-375	Levels were lower in subjects with diabetes before onset	Jiménez-Lucena et al.[37]
miR-31	Expression was downregulated in subjects with diabetic nephropathy, compared to that of diabetic patients without nephropathy	Rovira-Llopis ET AL.[38]
miR-126	Downregulated in patients with T2DM and nephropathy	Park et al.[39]
let-7d, miR-18a miR-18b, miR-23a, miR-27a, miR-28, miR-30d	The expression of these miRNAs decreased in non-progressive subjects with impaired glucose tolerance (IGT), compared to that of subjects who progressed to advanced T2DM	de Candia et al.[40]
miR-132, miR-29b, miR-223, miR-17-5p, Let-7b	The expression of these miRNAs was downregulated with increasing insulin resistance	Deiuliis.[27]
miR-26a, miR-126	The expression of these miRNAs was downregulated in subjects with diabetes in comparison to that of subjects without diabetes.	Jansen et al.[41]
miR-126	Expression was downregulated in subjects with T2DM and associated complications, compared to that of normoglycemics or subjects with pre-diabetes	Rezk et al.[42]
miR-126-5p	Levels were downregulated in subjects with coronary artery disease	Li et al.[43]
miR-320a, miR-197-3p, miR-23-3p, miR-27a-3p, miR-130a-3p	Levels of miRNAs were downregulated in individuals with metabolic syndrome and obese subjects	Goguet-Rubio et al.[44]

Table-II. Down-regulated miRNAs in diabetes mellitus in literature

The information from epigenetic studies highlight the fact that certain triggers disrupt the methylation status and miRNA signatures in the intronic regions, and subsequently modify gene expression. The outputs from these epigenetic changes are translated in various forms, which are interpreted as metabolic diseases, including cardiovascular diseases (CVDs), hypertension, and T2DM. Although the available data are preliminary, once the changes are interpreted by biotechnology tools, including biomarkers, such as miRNAs, the resulting data can serve as potential arsenals for predicting metabolic diseases, and can also be employed for targeting the concerned pathways for genomic medicine. We believe that more high-quality studies are necessary for employing the precise use of miRNAs as biomarkers for addressing the different stages of glycemia, ranging from normoglycemia to pre-diabetes to the onset of microvascular and macrovascular complications. The consolidated data obtained from this literature review are presented in Figure-2 for further evaluation.

The review has certain limitations: Firstly, most of the articles considered herein appear to be comparative cross-sectional studies, and there were a limited number of studies that specifically focused on human miRNAs in various stages of progression of T2DM. Secondly, most of the studies evaluated PCR techniques or equivalent strategies for analyzing the expression of miRNAs from the blood, and only a few studies reported further investigation by sequencing or microarray techniques. It therefore appears that more miRNAs might be identified for the diagnosis of T2DM and the associated complications when more advanced sequencing strategies are employed.

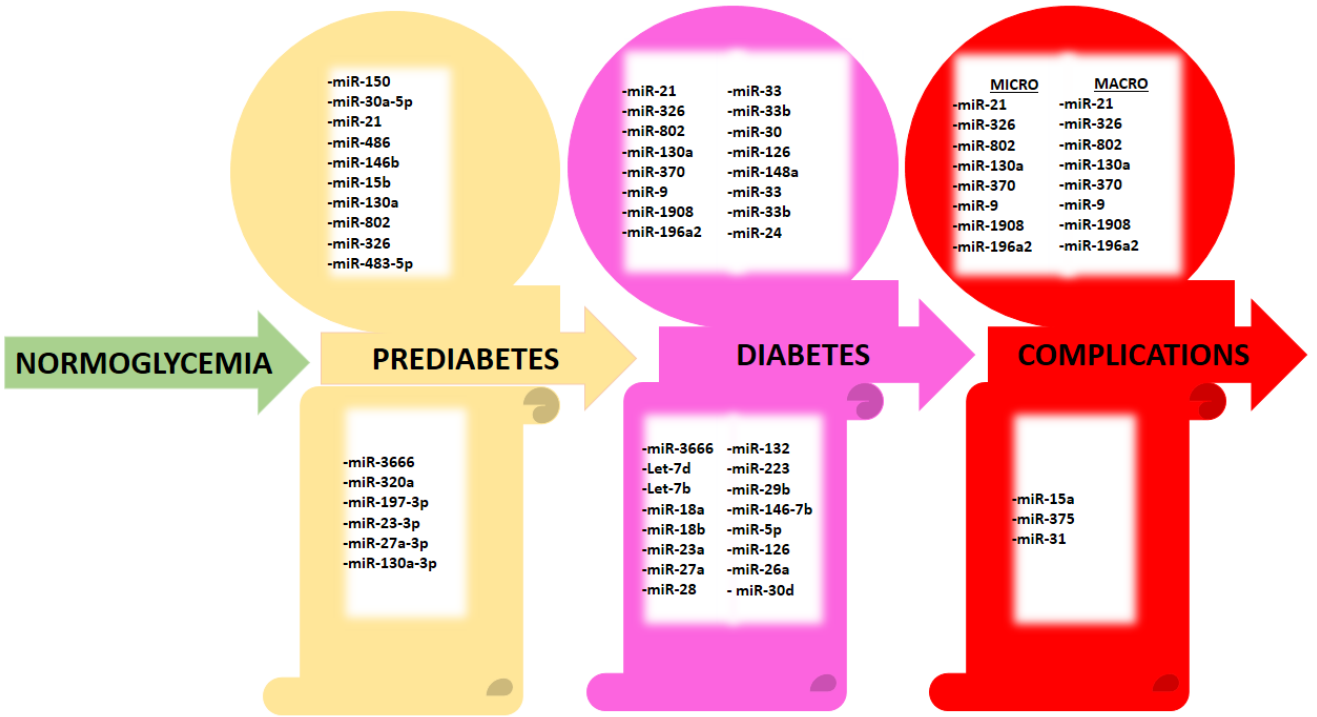

Figure-2. Consolidated overview of the research outcome on the various upregulated and down regulated clusters of miRNAs across various stages of glycemic dysfunction.

Lastly, the role of miRNAs as positive and negative acute phase reactants needs to be evaluated as the underlying alterations in metabolism-related inflammation might have interfered with the true evaluation of T2DM. This is attributed to the fact that certain miRNAs participate in inflammatory responses, and may be common to that of other similar diseases.

Despite the concern regarding the data reviewed herein, it is still possible to state that this review is one of the preliminary initiatives to define a T2DM signature from the literature presently available on this subject. The review can be improved over time with data from high-quality trials and results obtained using advanced sequencing technology. It is possible to conclude that research may further progress toward "RNA interference" technologies, which are primarily adopted for gene knockout therapies, and can be the final goal behind understanding miRNA patterns in various stages of T2DM.

CONCLUSION

miRNA as epigenetic marker were found to be varying in subjects with or without diabetes and across various stages of T2DM, showing up regulation and down regulation as highlighted in reviewed literature. We suggested a T2DM miRNA signature which may be helpful in further research and can provide a consolidated approach in clinical use. However, we feel further high quality research is needed to validate this data with control trials and using improved sequencing techniques.

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