

## MicroRNAs in hepatic pathophysiology in diabetes

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miRNAs that play a role in the altered hepatic behavior during diabetes.

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### Abstract

MicroRNAs (miRNAs or miRs) are small approximately 22 nucleotide RNA species that are believed to regulate diverse metabolic and physiological processes. In the recent past, several reports have surfaced that demonstrate the role of miRNAs in various biological processes and numerous disease states. For a disease as complex as diabetes, the emergence of miRNAs as key regulators leading to the disease phenotype has added a novel dimension to the area of diabetes research. On the other hand, the liver, a metabolic hub, contributes in a major way towards maintaining normal glucose levels in the body as it can both stimulate and inhibit hepatic glucose output. This equilibrium is frequently disturbed in diabetes and hence, the liver assumes special significance considering the correlation between altered hepatic physiology and diabetes. While the understanding of the mechanisms behind this altered hepatic behavior is not yet completely understood, recent reports on the status and role of miRNAs in the diabetic liver have further added to the complexities of the knowledge of hepatic pathophysiology in diabetes. Here, we bring together the various

### INTRODUCTION

"In diabetes, the thirst is great; for the fluid running off dries the body.... For the thirst, there is need of a powerful remedy, for in kind it is the greatest of all sufferings; and when a fluid is drunk, it stimulates the discharge of urine...."<sup>[1]</sup>

These are the words of one of the most celebrated physicians of ancient Greece, Aretaeus of Cappadocia. He went on to say that "a person suffering from diabetes leads a life that is disgusting and painful, followed by a speedy death". The term diabetes also seems to have been introduced into medical nomenclature by Aretaeus himself. A notable Roman physician, Galen, regarded diabetes as a disease of the kidneys, or as he put it, "diarrhoea of the urine"<sup>[2]</sup>. Several hypotheses followed thereafter and it was only after the 1500s that some of the intricate explanations of this complex disorder became known to mankind. In 1674, Thomas Willis<sup>[3]</sup> first differentiated diabetes from other causes of polyuria by the sweet taste of diabetic urine and also suggested that this sweetness

first appeared in the blood. Later, Matthew Dobson established that the sweetness of urine was due to sugar and the sweet component was later on identified precisely as glucose by Eugene Chevreul<sup>[2]</sup>. Thus, it was due to the keen observational skills of these early researchers that we started gaining some understanding of diabetes.

In the present day, diabetes and its mechanisms are much more understood although several complexities still exist. A contributing factor to this has been the fact that, in recent times, there has been a tremendous increase in the number of individuals affected by diabetes together with varied mechanisms of the disease manifestation. Its exploding trend is evident from world-wide predictions that forecast a number as high as 366 million patients with diabetes in 2030<sup>[4]</sup>. Mainly classified as two types, diabetes mellitus can be due to either insulin deficiency (due to loss of insulin producing pancreatic  $\beta$ -cells) referred to as type 1 diabetes mellitus, or due to decreased responsiveness of the body tissues to insulin referred to as type 2 diabetes mellitus (T2DM). The skeletal muscle, adipose and liver mainly comprise the insulin target tissues that together contribute towards maintaining a circulating euglycemic status in the presence of insulin. While the adipose tissue and the skeletal muscle majorly participate in sequestering glucose into the cells, the liver critically regulates hepatic glucose output by controlling the pathways of gluconeogenesis, glycolysis and glycogenolysis. In addition, the liver also communicates with other tissues and thereby regulates metabolism in extra-hepatic tissues, such as adipose and muscle. Therefore, the liver is believed to be a central organ in the regulation of whole body glucose homeostasis and is central to the onset and progression of diabetes. In this article, we discuss the hepatic abnormalities during diabetes and role(s) of microRNAs (miRNAs or miRs), the recently discovered small RNA species.

## LIVER IN DIABETES

The role played by liver in whole body metabolism and its implication in the pathogenesis of T2DM becomes even more significant as this tissue is sensitive to the two important hormones involved in glucose homeostasis, namely insulin and glucagon. Any disturbance in the delicate equilibrium maintained by the liver often leads to abnormal glucose levels within the body<sup>[5]</sup>. Moreover, owing to the hepatic portal system, the liver has an upper edge in encountering any changes in the nutritional status of an individual. Any manipulation in its physiology involving the intricate regulation of glucose homeostasis by alternating between cycles of glucose output and its inhibition contributes to the onset and progression of diabetes. Hence, it is not surprising that the liver is one of the affected and/or contributing tissues in diabetes mellitus.

One of the most prominent and evident symptoms of T2DM is a fatty liver. While adipocytes are normally believed to accumulate fats, under abnormal conditions (such as over nutrition), other organs like the liver and

muscle become additional sites for fat deposition. This “ectopic” fat accumulation causes several derangements, not only in the normal functioning of the organ involved but also in the whole body metabolism. One such abnormality that is evident in the liver during T2DM is non-alcoholic fatty liver disease (NAFLD).

NAFLD is a broad term comprising of liver disorders which are usually related to insulin resistance, metabolic syndrome or type 2 diabetes. NAFLD starts with hepatic steatosis that is characterized by fat accumulation in the hepatocytes. This fat accumulation is thought to be caused by metabolic imbalances such as higher amounts of dietary lipids, increased trafficking of free fatty acids from adipose to liver and increased *de novo* lipogenesis. This may also result from reduced fatty acid oxidation or impaired triglyceride secretion from liver *via* VLDLs. Hepatic steatosis might progress to a more severe form of NAFLD i.e., non-alcoholic steatohepatitis (NASH). In some cases, this condition may worsen, progressing to fibrosis (activation of hepatic stellate cells resulting in collagen deposition) and cirrhosis and finally, might also end up in hepatocellular carcinoma<sup>[6]</sup>.

The underlying molecular mechanisms that lead to such hepatic abnormalities during diabetes are far from being completely understood. The association among insulin resistance, accumulation of triglycerides in the liver and diabetes is quite well established<sup>[7]</sup> but the precise mechanistic events are still a matter of debate. The contribution of genes, urban lifestyle and intra-uterine environment in making us prone specifically to T2DM and its complications are understandable, but still are not completely sufficient to explain these mentioned phenomena. As researchers from diverse fields of genetics, epigenetics, molecular biology and evolutionary biology are still struggling to address these intriguing questions, the emergence and identification of microRNAs as new regulators in this disease have added a whole new layer of complexity. The next section focuses on the established role(s) of various miRNAs during the onset and progression of hepatic abnormalities.

## MICRORNAS AND THEIR ROLE IN THE DIABETIC LIVER

MicroRNAs represent a new class of small RNA molecules with an ability to fine-tune gene expression. These are endogenous, single-stranded approximately 22 nucleotide RNA molecules that have been identified in over 80 species, including those encoded by viral genomes<sup>[8]</sup>. They predominantly regulate target gene expression by binding to the 3'UTRs of the respective mRNAs (messenger RNA) and inhibiting their translation into the respective proteins. It is believed that expression of 30% of human genes may be regulated by miRNAs<sup>[8]</sup>.

In the last few years, several reports mentioned the association of miRNAs with diverse metabolic pathways, especially in the liver, that lead to the diabetic phenotype<sup>[9]</sup>. These studies not only underscore the fact that

these tiny RNA species are big players in hepatic pathophysiology during diabetes but also categorically emphasize that this field is growing quite rapidly. In one such study, a set of 18 distinct miRNAs were differentially expressed in the livers of hyperglycemic Goto-Kakizaki (GK) rats as compared to normal Brown Norway rats<sup>[10]</sup>. In particular, specific miRNAs namely miR-195 and miR-103 that exhibit highest levels of expression in the livers of the diabetic GK rats followed an almost linear co-relative expression pattern with the hyperglycemic phenotype. Also, miR-191 was significantly up regulated in GK rats as compared to other strains. Predicted targets to these altered miRNAs are involved in pathways pertaining to T2DM and therefore suggest the relevance of an altered miRNA status in the diabetic liver.

miRNAs were shown to play regulatory roles in the aberrant energy status during NAFLD. In a comparative study in ob/ob mice (a well established mouse model for diabetes with NAFLD) *vs* streptozotocin (STZ)-induced diabetic mice (this mouse model exhibits hyperglycemia without fatty liver), miR-107, miR-103, miR-126-3p, miR-100 and miR-29c were identified to be differentially expressed. On the other hand, miR-34a was specifically up regulated in STZ-induced diabetic mice in contrast to normal mice<sup>[11]</sup>. Such distinct patterns of miRNA expression identify an important role of miRNAs in the altered energy metabolism and pathophysiology of the liver during T2DM. Livers of ob/ob mice also exhibit elevated levels of miR-103/107 levels<sup>[12]</sup> and in an attempt towards reconfirming this in humans, patients with alcoholic liver disease, NAFLD and NASH also depicted increased levels of these miRNAs. Interestingly, antagonism of miR-103/107 in ob/ob mice resulted in increased insulin sensitivity and improved glucose homeostasis while gain of miR-103/107 function led to impaired glucose homeostasis. One of the mechanisms described for these miRNAs is *via* targeting Caveolin-1 that is involved in the activation of insulin signaling. Caveolins comprise a family of integral membrane proteins that are major components of membrane invaginations called caveolae. They are involved primarily in receptor-mediated endocytosis and caveolin-1, in particular, modulates insulin signaling by regulating glucose uptake<sup>[13,14]</sup>. miR-103/107, by targeting caveolin-1, therefore appears as a key regulator of insulin sensitivity and identifies a new target for the treatment of T2DM.

In addition to the above mentioned reports<sup>[9-12]</sup>, there are an increasing number of studies focusing on miRNA patterns in various stages of fatty liver disease. One of the earliest reports that came along in this area was by Jin *et al*<sup>[15]</sup>. The authors identified the differential miRNA expression patterns in liver among different stages of NAFLD using fat rich-diet based rat models. By applying prediction analysis of Microarray, miRNA signatures were identified specific to the stages of simple steatosis and steatohepatitis as compared to the normal liver. This, the first of its kind of study, provided not only an evidence for miRNA deregulation in fatty liver disease but also

suggested that miRNA signatures can be applied as diagnostic markers to differentiate among various pathological states of the liver. While miR-122, miR-27a, miR-31, miR-451, miR-145 were down-regulated, miR-200a, miR-200b were up-regulated in diet-induced NAFLD rat models<sup>[16]</sup>. Pputative targets of these altered miRNAs are involved in specific relevant pathways of lipid and carbohydrate metabolism, signal transduction and apoptosis. They also exhibited an inverse pattern of expression and this suggests a potential involvement of miRNAs and their target proteins in the pathogenesis of diet-induced NAFLD. Differential miRNA expression profiling in the hepatic tissues of patients of NASH identified a total of 46 miRNAs to be deregulated in these subjects<sup>[17]</sup>. Of these, silencing and over expression of miR-122 (one of the down regulated miRNAs) could regulate the expression of its predicted target genes mainly those involved in the lipogenic pathway (FASN, HMG-CoA reductase, SREBP-1c and SREBP-2), suggesting modulation of the hepatic fatty acid metabolism *in vivo*. Incidentally, an altered miRNA expression pattern in the livers of diet-induced mouse models for alcoholic as well as NASH was reported by Dolganiuc *et al*<sup>[18]</sup>, suggesting specific miRNA signatures in these hepatic pathologies even though both these abnormalities share common phenotypes.

In a rat model of hepatic fibrosis, the miR-34 family that targets ACSL1 and modulates lipid metabolism is altered<sup>[19]</sup>. ACSL1 belongs to the long-chain fatty acid CoA ligase family that activates long chain fatty acids to acyl-CoAs and hence by modulating lipid and fatty acid metabolism also contributes to the development of hepatic fibrosis. In patients of chronic hepatitis, the hepatic levels of miR-199a, miR-199\*, miR-200a and miR-200b are markedly elevated and also exhibit a positive correlation with the progression of the disease<sup>[20]</sup>. Fibrosis related genes, specifically TIMP1 and matrix metallo-proteinase 13, are increased in the presence of these miRNAs that lead to the development of hepatic fibrosis. All these suggest a diverse array of miRNAs getting altered during hepatic fibrosis and suggest their potential in therapeutic interventions.

Vinciguerra *et al*<sup>[21]</sup> described that unsaturated fatty acids increase the levels of miR-21 in hepatocytes, which in turn targets phosphatase and tensin homolog (PTEN), leading to steatosis. PTEN is a ubiquitously present protein that specifically dephosphorylates phosphatidylinositol (3,4,5)-trisphosphate and inhibits the AKT signaling pathway. This makes it important for the insulin signaling pathway of which AKT is a major intermediate and therefore the role of decreased PTEN expression has been well correlated with insulin resistance and fatty liver<sup>[22]</sup> but its regulation through miR-21 has further added a new mechanistic layer of regulation. The insulin-AKT axis and glucose homeostasis is also targeted by elevated miR-143 levels in the obese liver<sup>[23]</sup>. Over-expression of miR-143 impairs insulin-stimulated AKT activation that confers insulin resistance in obese mice. miR-143 does so by targeting oxysterol-binding protein related protein 8



(ORP8) that regulates the ability of insulin to phosphorylate and activate AKT. ORP8 is a member of the oxysterol-binding-protein family that binds 25-OH cholesterol and regulates the activation of AKT<sup>[23]</sup>. In a steatotic LO2 cell line model, miR-10b is up regulated that lead to steatosis *via* regulating its target PPAR $\alpha$ , a nuclear hormone receptor that is essential during lipid metabolism<sup>[24]</sup>. This interaction results in modulation of lipid metabolism by triggering the expression of genes required for fatty acid oxidation as well as by increasing the translocation of fatty acids into mitochondria. In another study, miR-27a and miR-27b were identified to be critical in the trans differentiation of normal quiescent HSCs (Hepatic Stellate Cells) into activated HSCs which play a significant role in liver fibrosis<sup>[25]</sup>. These miRNAs were able to modulate fat metabolism in HSCs by targeting RXR $\alpha$ , the expression of which is decreased in activated HSCs. As compared to normal quiescent HSCs, miR-335 is down-regulated in activated HSCs that lead to increased expression of matrix proteins,  $\alpha$ -SMA and collagen type 1<sup>[26]</sup>. Tenascin C, an extracellular matrix glycoprotein involved in cell migration, is down-regulated by miR-335 and the miR-335-tenascin C pair is identified, at least in part, to regulate HSC migration during hepatic fibrosis. Inhibition of HSC activation and migration is considered an effective therapeutic strategy and here miR-335 offers a promising treatment scheme for hepatic fibrosis.

Altered miRNA levels, therefore, are believed to determine the deregulated hepatic physiology that is frequently associated with T2DM and promise to be significant targets for therapeutic intervention.

## CONCLUSION

Current emerging studies indicate that miRNAs might be perceived as potential therapeutic targets. That this could be so is evident from reports where miRNA over-expression or antagonism led to regression of tumors in animal models<sup>[27,28]</sup>. These studies provide a proof of principle for the use of miRNAs in medicine. Since miRNAs exhibit partial complementarity with their target mRNAs, multiple mRNAs are targeted by each miRNA for example, a miRNA might target several components of a particular molecular pathway. miRNA based therapy might involve miRNAs, their mimics or their inhibitors known as antagomiRs to modulate the levels of the corresponding miRNA and their target(s) within a cell. So, miRNAs can be treated as multi-target therapeutic agents that might prove far more efficient than targeting a single gene or protein. However, a major obstacle envisaged is the targeted delivery and maintaining the stability of the miRNAs or their antagomiRs *in vivo*. Lipid conjugation and chemical modifications, especially additions of phosphorothioate, 2'-O-methyl, 2'-O-methoxyethyl moieties and locked nucleic acid motifs to the nucleotide backbone are being considered to overcome these obstacles<sup>[29]</sup>. These are believed to increase the target affinity and also enhance the serum stability of these small RNA

species. Indeed, *in vivo* inhibition of liver specific miRNA, miR-122 led to improvement in hepatic steatosis by increasing fatty acid oxidation and down regulating lipogenesis in diet-induced obese mice<sup>[30]</sup>. It also resulted in decreased cholesterol levels. Their therapeutic potential is also apparent in a recent study by Trajkovski *et al*<sup>[12]</sup> where miR-103/107 antagonism led to improved insulin sensitivity and restored glucose homeostasis in obese mice.

In addition, miRNAs can be utilized as superior biomarkers for diagnosis of various diseases as well as distinguishing different forms of the disease. Diagnosis remains a major unresolved issue related with NAFLD<sup>[6]</sup>. To date, there is no gold standard technique to either identify NAFLD or to differentiate among different stages of this disease. Serological assays based on conventional liver tests for alanine aminotransferase and aspartate aminotransferase and imaging techniques such as hepatic ultrasonography, magnetic resonance imaging and proton magnetic resonance that are routinely used for the detection of NAFLD have their associated shortcomings. Some recent studies have shown that different types of cancers can be discriminated by their specific miRNAs expression signatures<sup>[27]</sup>. Also, presence of miRNAs in body fluids such as serum and increased stability as compared to mRNAs since they circulate within membrane vesicles which protect them from endogenous RNase activity<sup>[31]</sup>, make them even more attractive agents as biomarker diagnostics. In fact, circulating miRNA profiles have been explored as potential non-invasive biomarkers in several diseases<sup>[32]</sup>. Plasma levels of cardiac-specific miR-208 and miR-499 are elevated in acute myocardial infarction<sup>[33,34]</sup> and in a mouse model of drug-induced liver injury, miR-122 and miR-192 are specifically elevated<sup>[35]</sup>. Reduced levels of plasma miR-132 are associated with rheumatoid arthritis and osteoporosis<sup>[36]</sup>. Circulating miRNA levels have been most identified in various types of cancer; plasma miR-21 levels are elevated in B-cell lymphoma patients<sup>[37]</sup> and in leukemia patients, miR-92a levels are down-regulated<sup>[38]</sup>. Serum miR-141 levels are elevated in prostate cancer patients<sup>[39]</sup> and the miR-17-92 cluster is associated with colon cancer<sup>[40]</sup>. These reports suggest that circulating miRNAs are altered in diverse disease states and might be explored as potential biomarkers to identify the stage and progression of the disease.

Not much has been done as far as the circulatory miRNA profiles in T2DM or hepatic pathophysiology are concerned. Identification of altered circulatory levels of miRNAs in T2DM is just at the beginning<sup>[41-43]</sup> but nevertheless suggest a promising aspect of their use as potential non-invasive biomarkers in T2DM. Although not in relation to diabetes yet, plasma levels of miR-122, miR-34a and miR-16 were markedly elevated in NAFLD subjects that also correlated with the disease severity<sup>[44]</sup>. Therefore, although at its nascent stage, an altered miRNA signature offers a strong possibility to be exploited to identify hepatic abnormalities and also the stage of the disease during T2DM. Such a therapeutic potential of miRNAs in targeting the challenging issues of obesity

and T2DM has recently been described by Czech *et al*<sup>[29]</sup> in 2011.

Considering the increasing body of evidence that reveal altered hepatic miRNA patterns in diabetes, a worthwhile thought would be to utilise these small RNA species in the diagnosis and prognosis of this abnormality that would put forth novel criteria for their clinical identity.

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