Micro-ribonucleic acid modulation with oxidative stress and inflammation in patients with type 2 diabetes mellitus – a review article

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Abstract

In parallel with the rapid growth of obesity, there is also an increase in the prevalence of type 2 diabetes mellitus (T2D) worldwide. Due to its complications, cardiovascular diseases are the leading cause of death in those patients. In the last two decades, special attention has been given to oxidative stress and inflammation, as the underlying mechanisms related to T2D occurrence and progression. Moreover, micro-ribonucleic acids (miRNAs) as new genetic biomarkers take an important place in the investigation of different metabolic pathways of insulin signaling. In this review article, we discuss microRNA modulation with oxidative stress and inflammation in patients with T2D. Better insight into the novel potential therapeutic targets for treatment of diabetes and its complications is of utmost importance for public health.

Key words: diabetes, inflammation, micro-ribonucleic acids, oxidative stress.

Introduction

The prevalence of type 2 diabetes mellitus (T2D) is rapidly increasing worldwide, mostly as a consequence of the increasing number of individuals with obesity, unhealthy dietary patterns, as well as sedentary lifestyles. Individuals with obesity (i.e., with body mass index (BMI) > 35 kg/m^2) are 20 times as likely to get T2D as compared to normal weight subjects (i.e., 18.5 kg/m^2 < BMI < 25 kg/m^2) [1]. Insulin resistance is the common background of many metabolic pathways even before manifest diabetes occurs. Indeed, many metabolic disorders such as visceral obesity, metabolic syndrome, and non-alcoholic fatty liver disease share the common denominators, i.e. insulin resistance and concomitant dyslipidemia, that are the risk factors for T2D [2–6]. Even then, when the diagnosis of diabetes is established, much more has to be done in order to monitor its progression and to prevent and/or delay its complications or even death. In line with this, cardiovascular disease (CVD) still represents the cause of death in nearly 80% of T2D patients [1].

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In order to gain deeper understanding of the not so simple insulin signaling pathways, many novel biomarkers including genetic biomarkers named micro-ribonucleic acids (miRNAs) that are involved in their regulatory mechanisms are emerging nowadays, with special attention paid to oxidative stress and inflammation [2, 3, 7–11].

Oxidative stress and inflammation in type 2 diabetes mellitus

Oxidative stress or redox imbalance is a condition (phenomenon) that occurs when the factors of oxidative stress – prooxidants (free radicals) – overcome the mechanisms of antioxidant protection. Homeostatic mechanisms ensure the balanced production of free radicals and a whole series of antioxidants that are responsible for their safe removal. Free radical formation is a part of the physiological processes in a healthy organism, and since some of these reactive species are specific signaling molecules their presence and activity are necessary. However, in various pathological conditions such as cancer, CVD, diabetes, autoimmune diseases, rheumatic diseases, systemic lupus, and various skin diseases, the formation of free radicals overwhelms the defense mechanisms and develops "oxidative stress" which damages cells and tissues. Oxidative stress intensification can also occur due to the reduced efficiency of the antioxidant protection system, whose individual elements can be damaged during various pathological processes. Increased production of free radicals and a decrease in antioxidant protection most often run in parallel as part of pathophysiological processes, so oxidative cell damage grows, which complicates the distinct disease course that certainly takes place with many other mechanisms [12–14].

Oguntibeju defined T2D as a disease in which the intersection of hyperglycemia, oxidative stress, and inflammation occurs, and suggested that the main reason for the disease progression could be oxidative stress induction of inflammation and vice versa [15]. Low-grade inflammation (subclinical) contributes to diabetes development by amplifying insulin resistance and is also connected with hyperglycemia.

Obesity as a risk factor for T2DM is also a source of pro-inflammatory proteins generated through the activation of two pathways: stress-activated Jun N-terminal kinase (JNK) and nuclear factor κB (NF-κB) [16, 17]. Inflammatory agents considered as the most important for diabetes progression are TNF-α, interleukins (IL-1, IL-6, IL-10), adipokines (leptin, adiponectin, resistin), monocyte chemoattractant protein, angiotensin, and serum amyloid A [18]. The initial generation of inflammatory biomarkers causes adipose tissue infiltration by macrophages and immune B and T cells, which in turn initiates long-standing low-grade inflammation, constantly producing more cytokines [19]. The vicious circle of unwanted events increases the type and level of cytokines, which presents a link between obesity, insulin resistance, and inflammation [20].

The study of Stefanović *et al.* regarding oxidative stress and patients with T2D showed a significant increase in plasma levels of thiobarbituric acid reactive substances (TBARS), superoxide anions (O_2^-), and superoxide dismutase (SOD), and a lower sulfhydryl group concentration compared to healthy subjects. Moreover, the study reported that obese T2D subjects had worse redox balance than normal-weight patients [21]. The second arm of the study analyzed the relationship between cardiovascular risk score and plasma oxidative stress marker levels in T2D patients and revealed an association of cardiovascular risk score with oxidative stress, indicating superoxide anion involvement in CVD development [22]. The third arm of the same study found reduced paraoxonase 1 (PON1) activity and impaired lipoprotein metabolism of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) particles in T2D patients compared to healthy subjects [23]. Wu *et al.* in their meta-analysis detected a relationship between low PON1 activity and the risk of T2D, diabetic macroangiopathy, and microangiopathy, i.e. the role of PON1 activity in progression of diabetic complications [24]. Pandey *et al.* [25] suggested that oxidative stress in obese diabetic subjects induces hyperleptinemia which leads to irregular leptin signaling, i.e. leptin resistance, which deprives leptin of its protective effects, and insulin resistance develops in these patients. Klisic *et al.* [26, 27], trying to connect oxidative stress, inflammation, and dyslipidemia in patients with T2D with fatty liver disease, concluded that only a multimarker approach, including mentioned biomarkers, could be beneficial in predicting the risk of developing this serious comorbidity. The significant relationship between increased xanthine oxidase activity and uric acid concentration with albuminuria progression in T2D patients undoubtedly links oxidative stress exacerbation with kidney disease as a chronic diabetes complication [28]. Furthermore, another study confirmed the relationship between glycated hemoglobin (HbA$_{1c}$) levels and a comprehensive risk score consisting of biomarkers of dyslipidemia, oxidative stress, and inflammation in both prediabetic patients and T2D patients [29]. A significant positive correlation between HbA$_{1c}$ and total sulfhydryl group concentration in the general population might be explained by increased production of antioxidants generated in an attempt to diminish increased

levels of glycemia-induced reactive oxygen species (ROS) formation [30]. Similarly, Kohata *et al.* observed a correlation between glycated albumin and oxidative stress in patients with well and poorly controlled T2D [31].

Micro-ribonucleic acids

Since the last decade of the previous century, there has been extensive research on new genetic biomarkers named micro-ribonucleic acids (miRNAs) [32]. They are short single-stranded molecules with a length of 19 to 25 nucleotides with an originally assigned role in gene expression regulation [11]. They do not code proteins, but with their seed sequence, a region centered on nucleotides 2–7, they bind to 3′-untranslated regions (3′UTR) of messenger RNA (mRNA), causing its degradation and destabilization or translation repression. The fate of mRNA depends on the degree of complementary superposition between it and the miRNA [33]. When mRNA and miRNA perfectly match, mRNAs are cleaved and degraded. However, when binding is imperfect, the complex miRNA inhibits mRNA translation [34]. One single miRNA may hybridize to multiple target sites on more than 100 mRNAs [35]. Like mRNAs, miRNAs are transcribed from genomic DNA in the nucleus in the form of long precursor molecules several kilobases in length called primary miRNA transcripts (pri-miRNA) by the action of RNA polymerase II. Pri-miRNAs are further transformed into smaller molecules around 100 nucleotides in length by the action of the RNA polymerase III enzyme Drosha [36]. When formed, these pre-miRNAs are transferred into the cytoplasm by exportin 5. Single-stranded mature miRNAs are then synthesized by the action of the RNase III-type endonuclease Dicer. By itself, miRNA cannot perform its biological effects but with RNA binding proteins (RBPs) it forms the miRNA-induced silencing complex (miRISCs) in which it is delivered to and may bind to its mRNA targets [37].

Besides translation repression, certain miRNAs may also participate in gene activation. This effect may occur via direct activation of target mRNA, its stabilization, and translational activation [38].

In normal physiological states, miRNAs are involved in homeostatic metabolic regulations and development processes. In recent years, studies have proved that due to genetic and environmental factors, miRNAs might be dysregulated, playing a significant role in the development and progression of many diseases such as cancers [39], CVD [40], T2D [41], and others [42].

The aberrant miRNA expression may have potential roles in underlying pathophysiological mechanisms of T2D development and progression, which include β-cell function and development, insulin production and secretion, insulin resistance, glucose and lipid metabolism, inflammation, and oxidative stress [43]. This review will summarize some of the ones implicated in these processes.

miR-375 is one of the most studied miRNAs regarding T2D. It originates from the pancreas [44]. Also, it is expressed by the brain and spinal cord [45]. Its main roles primarily rely on establishing the normal endocrine pancreatic mass and maintaining glucose homeostasis [44]. miR-375 directly targets transcripts of genes that negatively regulate cell growth and proliferation of β-cells [44]. miR-375 downregulates 3-phosphoinositide-dependent protein kinase-1 (PDK1) directly interacting with the a3′UTR of its mRNA [46] and suppresses PDK1 protein production. PDK1 is an important participant in insulin signaling inducing lipogenesis and GLUT-4 translocation. miR-375 has a biological role as an inhibitor of phosphoinositide 3-kinase (PI3K) signal transduction via PDK1 [46]. By the PI3K cascade, it controls insulin gene expression in pancreatic β-cells and its proliferation [47]. By inhibition of myotrophin (Mtpn), miR-375 inhibits insulin release from β-cells [44]. Furthermore, Mtpn-mediated upregulation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), associated with improved insulin secretion stimulated by insulin, is inhibited by this miRNA [48, 49]. By indirect inhibition of NF-κB, a master transcription factor regulating pro-inflammatory and oxidative stress-related genes, miR-375 blocks their detrimental effects [44, 48]. Prolonged glucose exposure of INS-1E cells (β-cell model) leads to decreased miR-375 levels and increased insulin receptor substrate 2 (IRS2) gene expression and protein kinase B (PKB, also known as AKT kinase) and glycogen synthase kinase 3 (GSK3) phosphorylation [46]. The last two both act downstream of PDK1 [46].

Family members of the miR-29 gene (miR-29a, miR-29b, and miR-29c) participate in glucose and lipid metabolism regulation during T2D development and progression [50]. These isoforms are abundantly present in β-cells and might contribute to β-cell development and functions [51]. These family members have effects in insulin release by decreasing levels of the Onecut2 (OC2) transcription factor, which in turn stimulates the expression of granuphilin, which further inhibits insulin release, promoting apoptosis [52]. miR-29a and miR-29b specifically target mRNA of plasma membrane's monocarboxylate transporter (Mct1) and decrease its protein levels. When present at a lower level it leads to a decreased cytosolic ATP/ADP ratio and consequently decreased insulin release from β-cells [51]. Hyperinsulinemia and hyperglycemia induce miR-29a and miR-29b, but

not miR-29c, gene expression in 3T3-L1 adipocytes [53]. miR-29 inhibits phosphorylation of PKB and subsequently its activity, and translocation of GLUT4 transporter to the cell surface after insulin stimulation. Also, phosphorylation and expression of IRS-1 are attenuated. Therefore, it participates in silencing components of the insulin signaling cascade [53].

The miR-124a gene family consists of three isoforms – miR-124a1, miR-124a2, and miR-124a3 – abundantly expressed by the brain and pancreatic β-cells. It exerts some of its actions in a synergistic manner with miR-375 [54]. It suppresses the translation of Mtpn and insulin release into the bloodstream. Via the transcription factor Forkhead box a2 (Foxa2), it regulates β-cell functions and physiology [55]. Upregulation of miR-124a leads to a decrease Foxa2 protein levels following lower gene expression of the two subunits Kir6.2 and Sur-1 of the ATP-dependent K (KATP) channel or pre-proinsulin, leading to lower insulin production and release [55], as well as to decrease of pancreatic and duodenal homeobox 1 (Pdx1), which influences β-cell differentiation and development [56]. miRNA-124a does not interact directly with these transcripts. It is more obvious that a decrease in their levels is the result of a cessation of transcriptional stimulation by Foxa2. Decreased Foxa2 worsens insulin resistance in peripheral tissues [55]. The miR124a increases neurotransmitters' (SNAP25, Rab3A, and synapsin-1A) mRNA and protein levels probably indirectly at the transcriptional level, whereas mRNA levels of the endocrine factors Rab27A and Noc2 were reduced due to their direct targeting by miR124a, which inhibits their translation [57]. An imbalance between neurotransmitters and endocrine factors appears to contribute to impaired insulin exocytosis from β-cells [57].

miR-9 is expressed in neurons and pancreatic β-cells originating from three different chromosomes: 1, 5 and 15 [58]. Its participation is obligatory for insulin exocytosis by two different mechanisms examined so far. It binds to transcription factor OC2, decreasing its translation and consequently the protein level, which cannot inhibit granuphilin expression and lowers glucose-stimulated insulin release [58]. Sirtuin 1 (SIRT1) mRNA is also a target for miR-9 and its translation is inhibited by this miRNA in pancreatic β-islets [59]. Also positive effects on insulin secretion and antioxidative status are suppressed because SIRT1 has antioxidative and anti-inflammatory properties [60].

miR-96 has a much broader expression pattern than miR9, miR124a, and miR375 [57]. Besides neurons and β-cells, miR-96 is expressed by the placenta [61], lungs [62], gastrointestinal tract [63], etc. Similarly as in the case of miR-124a, miR-96 inhibited the insulin exocytosis stimulated by glucose in MIN6 cells [57]. miR96 induces higher levels of granuphilin mRNA and lower amounts of Noc2 mRNA. Lovis et al. [57] determined that miR-124a exerted clearly more distinctive effects on the exocytotic machinery than miR-9 and miR-375. In contrast, miR-96 partially demonstrated similar effects as miR-9. miR-9 stimulates translational repression of OC2, which negatively regulates granuphilin transcription, but miR-96 affects OC2 mRNA levels via an indirect mechanism because the 3′UTR of OC2 mRNA does not contain its binding site. The same was demonstrated for the 3′UTR of Noc2 mRNA [57].

The miR-200 family encompasses members belonging to two polycistronic clusters, mir-141/200c on chromosome 12 and mir-200a/200b/429 on chromosome 1, regulating different target genes [64]. These are miR-141, miR-200c, miR-200a, miR-200b, and miR-429, expression of which is induced in β-cells of diabetic mice [65]. Mir-200a, -200c, and -429 were shown to be the main principal regulators of β-cell apoptosis. However, inhibition of all miR-200 family members protects β-cells from oxidative and endoplasmic reticulum (ER) stress and prevents apoptosis through anti-stress and/or pro-survival signaling pathways including induction of X-linked inhibitor of apoptosis (XIAP) and juxtaposed with another zinc finger protein 1 (JAZF1) genes [65]. The most upregulated family member is miR-200c, which promotes oxidative stress and ROS production and decrease nitric oxide (NO) interfering with the SIRT1/forkhead box O1 (FOXO1)/endothelial nitric oxide synthase (eNOS) signaling cascade [64].

miR-126 is mainly expressed by endothelial cells [66]. It was found to be involved in the development of insulin resistance in hepatocytes and myocytes. Mitochondrial dysfunction which occurs in an oxidative stress environment induces its expression [67]. MiR-126 directly binds to the 3′UTR of IRS-1 mRNA and inhibits its translation. Additionally, it binds to the PI3K regulatory subunit beta (p85β) and negatively regulates the PI3K-AKT signaling pathway, inhibiting GLUT4 translocation to the plasma membrane [68]. Oppositely to this, under high blood glucose miR-126 reduced the inflammatory reaction of human gingival fibroblasts via inhibition of tumor necrosis factor receptor associated factor 6 (TRAF6) in the NF-κB signaling pathway and decreased the secretion of IL-6, TNF-α, and C-C motif chemokine ligand 2 (CCL2) [69].

Family members of the miR-33 human gene (mir-33a and mir-33b) originate from chromosomes 22 and 17, respectively [70] within the intronic sequences of the sterol regulatory-element

binding protein (SREBP) genes. MiR-33a and -33b specifically inhibit the expression of 5′-adenosine monophosphate-activated protein kinase subunit-α (AMPKα), and IRS2 genes downregulating insulin signaling and fatty acid oxidation, respectively [71]. They also target genes involved in cholesterol export, the adenosine triphosphate binding cassette (ABC) transporters and the Niemann-Pick C1 (NPC1) transport protein [72]. miR-33 reduced insulin-stimulated AKT and ERK phosphorylation, leading to reduced IRS2 levels [71]. MiR-33b overexpression significantly lowers the sirtuin 6 (SIRT6) mRNA and protein levels in Huh7 cells [71] and increases glucose uptake with up-regulation of glycolysis. Protection of cells from DNA damage and oxidative stress could be reduced due to SIRT6 downregulation [73]. Also, this SIRT6 deficient environment increases HIF1α activity, diminishes mitochondrial respiration, which might increase oxidative stress, and activates NF-κB target genes [73].

In human studies, miR-375, miR-29, miR-124a, miR-9, miR-33a levels were significantly upregulated in the serum of patients with T2D compared to controls and were proposed to be significant predictors of diabetes [74]. Levels of miR-96 [75] and miR-126 [76] were downregulated in patients with T2D. However, only miR-375 and miR-9 were found to predict not only T2D but also prediabetes [77].

Relationship between micro-ribonucleic acids and redox status

Intracellular ROS perform a role in cellular signaling but only at low and intermediate concentrations, which enables control of cell proliferation, differentiation, energy balance maintenance, apoptosis, and the stress response [78]. ROS level in the cell is controlled by several transcription factors: Nrf2, AP-1, NF-κB, HIF-1, and p53. Transcription factors' activity induces antioxidative protection enzymes, which in turn neutralize ROS. Redox-sensitive proteins/enzymes (thioredoxin, glutaredoxin, peroxiredoxin) control redox homeostasis by exchange of reduced-oxidized sulfhydryl groups. Importantly, redox regulation is governed not just by the above-mentioned proteins, but also by recently revealed non-coding RNA sequences, whose function is directed towards particular gene silencing [79, 80]. Among several non-coding RNAs, microRNA (miRNA) has also a significant role in cellular redox homeostasis sustention [81]. It seems that the miRNA and oxidative stress relation is bidirectional. ROS could regulate miRNA biogenesis by downregulation of ribonuclease III (Dicer). Conditions of chronic hypoxia cause suppression of several miRNAs' expression [80]. NRF-2 and ARE (antioxidant-responsive elements) inducers could upregulate Dicer expression and thus miRNA syn-

thesis in animal models [82]. Leisegang *et al.* have shown that the influence on ROS on expression of different miRNAs could be in both directions, increasing and also decreasing, which is probably connected with experimental conditions [79]. It is already proven that some miRNAs could influence oxidative stress through the modulation of gene expression of antioxidant enzymes, for example SOD [80]. Since altered regulation of miRNA is associated with oxidative stress, the miRNA expression restoration to normal values may lead to a new therapeutic approach to prevent oxidative damage [83]. LaPierre *et al.* documented the influence of miRNAs on β-cell function in different metabolic conditions [84]. La Sala *et al.* found that miRNA-21 was upregulated in patients with impaired glucose tolerance and T2D patients as compared to normoglycemic subjects [85]. miRNA-21 correlated with increased ROS production (Spearman's ρ = 0.2, p = 0.008) and decreased antioxidants activity [i.e. with plasmatic SOD2 (ρ = -0.34, p = 0.002)]. MiRNA-21 inhibition succeeded to reverse its influence on expression of FOXO1 and SOD2 genes, which is diminished in diabetes [85]. Regarding T2D, it is interesting to pay attention to the miRNA-200 family, which is important for redox regulation and could be positively modulated by hydrogen peroxide (H$_2$O$_2$). Hyperglycemia leads to oxidant protein modification and formation of advanced glycation end products (AGE), which could be modified by different miRNAs [83]. MitomiRs are recently discovered miRNAs situated in mitochondria and are able to regulate mitochondrial function connected with fatty acid oxidation, which is also linked to oxidative stress [83]. Sun *et al.* observed redox status improvement by fruit juice (*Actinidia chinensis* Planch.) consumption in T2D patients. These effects occurred through Keap1 and Nrf2 activation by miRNA-424 upregulation, which results in SOD and glutathione (GSH) increase and the concomitant decrease in the pro-inflammatory cytokines interleukin (IL)-1 and IL-6 [86]. According to many investigations, it can be concluded that the miRNA network is mutually connected with redox status and this connection is some kind of feedback loop in which ROS regulates miRNA expression and the changes in miRNA expression regulate the fine balance between prooxidant and antioxidant factors.

Complications of type 2 diabetes mellitus

Complications of T2D are divided into macrovascular complications including coronary artery disease (CAD), stroke and peripheral artery disease (PAD) [87], and microvascular complications (DMC) of diabetic retinopathy (DR), nephropathy (DN), and peripheral neuropathy (PN) [88, 89]. The morbidity and mortality associated with diabetes mellitus are driven by the associated micro-

vascular and macrovascular complications, which contribute substantially to the cost of diabetes to health care systems. Data from the GRADE Study [90] suggest that the overall prevalence of T2D complications is higher than expected: 21.5% of participants developed peripheral neuropathy; 9.7% of them developed cardiovascular autonomic neuropathy and 1.0% developed retinopathy. Myocardial infarction was present in 7.3% of cases, and stroke in 2.0% of cases.

Micro-ribonucleic acids and macrovascular complications in type 2 diabetes mellitus

Pathological changes in endothelial cells (ECs), cardiomyocytes, and vascular and stem cells represent the hallmarks of macrovascular complications of T2D. Impaired insulin signaling, oxidative stress, and inflammation initiate endothelial dysfunction and rapidly promote atherosclerosis development in T2D patients [91]. Many miRNAs have important roles in the regulation of the immune system, inflammatory response, and platelet function, which are the key factors not only in atherosclerosis development but also in the clinical manifestation of the disease [92].

A panel of 3 plasma miRNAs (miR-21, miR-218, and miR-211) has been identified for early detection of atherosclerosis in patients with T2D, compared to healthy subjects and non-complicated T2D [93]. Downregulation of miR-223 and miR-146a expression in plasma and platelets was found in patients with T2D with and without ischemic stroke; however, there were no changes in non-diabetic patients with stroke compared to healthy controls, implying the specificity of these markers for T2D related complications. These two miRNAs target several genes implicated in platelet activation, so it was proposed that hyperglycemia might influence the expression of these miRNAs, leading to platelet activation and increased risk of ischemic stroke in T2D patients [94].

Patients with T2D and a prior history of cardiovascular events showed upregulated levels of pro-inflammatory miR-21 and down-regulation of miR-126 in circulating angiogenic cells compared to healthy subjects [95, 96]. Furthermore, upregulation of plasma miR-126 and miR-210 in T2D with CAD compared to T2D without CAD indicated that these miRNAs could serve as epigenetic biomarkers for CAD in T2D [97] while decreasing circulating miR-126 levels predicted patients with diabetic CAD [98].

Recently it was shown that exosomal miRNAs play an important role in the development of vascular endothelial disease related to diabetes macrovascular complications. The elevation in number of circulating endothelial microparticles (EMPs) derived from endothelial cells (EC) was related to atherosclerosis development in diabetes mellitus [99]. Jansen *et al.* reported that EMPs could transfer miR-222 into recipient ECs, where it promotes anti-inflammatory effects by inhibiting the expression of intercellular adhesion molecule (ICAM)-1 [100]. However, miR-222 was decreased in EMPs derived from high glucose damaged ECs, lowering their anti-inflammatory capacity, highlighting this pathway as a potential therapeutic target for CAD in T2D [101].

Micro-ribonucleic acids and microvascular complications in type 2 diabetes mellitus

An increased inflammatory response is a major factor in the development of DMC, triggered by hyperglycemia, hemodynamic and oxidative stress [102]. As a consequence, cell damage occurs, often leading to apoptosis, which in the case of terminally differentiated cells has irreversible effects on the tissue function [102]. For instance, in DR low grade inflammation worsens hyperglycemia-induced injury and neovascularisation [103]. Increased evidence suggests that that miRNAs are dysregulated both in human and experimental DMC [102, 104–127].

Given that inflammation is a common feature of all DMC [102], dysregulation of certain miRNAs involved in the regulation of the immune system and inflammatory response is found in all three microvascular pathological complications of T2D (DR, DN, and PN).

MiR-146a is upregulated by activation of the NF-κB pathway. However, following its upregulation, miR-146a downregulates interleukin-1 receptor-associated kinase 1/2 and TNF receptor-associated factor 6, which has a negative feedback effect and limits the proinflammatory action of NF-κB [102]. Therefore, it is not surprising that the expression levels of miR-146a in various human and animal models of DMC (kidney, retina, sciatic nerve) have varied from downregulation [104–106] to upregulation [107–111]. This implies that the overall effect of miR146a anti-inflammatory actions is depended on the stage and type of the complication development. MiR-21 is a proinflammatory miRNA, which was found to be upregulated in DR and DN [111–116], in that way contributing to increased inflammation and further progression of DN and DR. Upregulation of serum miR-518d-3p and miR-618 was found as a common feature of T2D with multiple microvascular complications in comparison to individuals with no microvascular complications [128].

Vascular endothelial growth factor (VEGF) plays a key role in hypoxia-induced abnormal neovascularization in DR. Its expression is influenced by several miRNAs, some of which directly target VEGF (miR-126, miR-106, miR-15, and miR-200b),

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while others indirectly modulate VEGF (miR-150, miR-184, and miR-155) [102]. For instance, the expression of miR-126 was downregulated in ECs and retinal pericytes from a DR mouse model. MiR-126 is identified as one of the main factors and potential therapeutic targets in DR, which acts upon IRS-1, leading to its upregulation, resulting in suppressed cell invasion and viability [117]. In the vitreous body tissues in patients with proliferative diabetic retinopathy (PDR) the expression level of miR-126 was significantly lower in the control group compared to stage IV, V, and VI groups, and negatively correlated with VEGF mRNA levels [118]. It was found that miR-126 reduces experimental DR and suppresses endothelial cell proliferation and migration by targeting polo-like kinase 4 [119], and that miR-126 stimulates proliferation and inhibits apoptosis in high-glucose-induced HRECs, through the PI3K–AKT pathway [129]. In addition, serum miR-126 levels had significant diagnostic value for PDR, with an area under the curve (AUC) of 0.976 with 81.21% sensitivity and 90.34% specificity in discriminating PDR from healthy individuals [130]. MiR-1281 was up-regulated in both serum of DR patients and in high glucose-cultured retinal cells, indicating that miR-1281 could be an important regulator in DR [120]. Recently, a meta-analysis revealed that the hsa-miR-126 family was significantly downregulated in blood from patients with DN, while its urinary level was upregulated, highlighting the potential importance of the hsa-miR-126 family in both pathogenesis and diagnosis of DN [131].

Furthermore, this meta-analysis showed that the hsa-miR-770 family was significantly upregulated in urine and blood samples from patients with DN [131].

Examination of individual renal biopsies confirmed that low levels of miR-192 were strongly correlated with low estimated glomerular filtration rate (eGFR) and high fibrosis score, indicating a reduction of miR-192 in advanced DN [121]. Furthermore, TGF-β-dependent loss of miR-192 was associated with tubulointerstitial fibrosis by downregulating E-cadherin with consequent promotion of epithelial-to-mesenchymal transition (EMT) in PTCs in HK-2 cells [121]. On the other hand, several studies have suggested that miR-192 might induce collagen expression by targeting ZEB, which repressed the E-box element in the enhancer region of the collagen gene [122, 123]. These inconsistencies between human and animal studies as well as different experimental settings highlighted the complexity of the regulatory roles of miR-192 in DN [124]. Guo *et al.* found that miRNA-29c was overexpressed in podocytes, having a proinflammatory effect. MiRNA-29c promoted the progression of DN by targeting tristet-raprolin, making it another potential target for the therapy of DN [125].

As already indicated, miRNAs involved in inflammatory processes through the NF-κB pathway in DR and DN inflammation reflect their effect in PN as well. Moreover, in neuropathic tissues, in long-term exposure to hyperglycemia, the release of cytokines such as IL-1β, MCP (monocyte chemoattractant protein)-1, and TNF-α occurs, alongside the involvement of miRNAs in this process [126]. Several other miRNAs were proposed to influence PN in animal models which are extensively reviewed by Simeoli *et al.* [89]. As for human studies, a study by Li *et al.* analyzed the differential expression of various genes in non-progressing and progressing DN nerve samples, and revealed three specific miRNAs associated with DN – miR-377, miR-216a, and miR-217 – which targeted 1052 genes, most of which were related to inflammation [127], highlighting possible therapeutic targets in diabetes-related neuropathy.

The better insight into pathophysiological mechanisms of onset and progression of diabetes, with special attention to oxidative stress and inflammation, as well as exploring the new genetic biomarkers, such as miRNAs that may have an impact on insulin signaling pathways, may lead us to novel potential therapeutic targets and discoveries for treatment of diabetes and its complications.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

References

1. Papaetis GS, Papakyriakou P, Panagiotou TN. Central obesity, type 2 diabetes and insulin: exploring a pathway full of thorns. Arch Med Sci 2015; 11: 463-82.
2. Klisic A, Kavaric N, Kotur-Stevuljevic J, Ninic A. Serum soluble transferrin receptor levels are independently associated with homeostasis model assessment of insulin resistance in adolescent girls. Arch Med Sci 2021; doi: https://doi.org/10.5114/aoms/132757.
3. Klisic A, Kavaric N, Jovanovic M, Soldatovic I, Gligorovic-Barhanovic N, Kotur-Stevuljevic J. Bioavailable testosterone is independently associated with fatty liver index in postmenopausal women. Arch Med Sci 2017; 5: 1188-96.
4. Kavaric N, Klisic A, Ninic A. Are visceral adiposity index and lipid accumulation product reliable indices for metabolic disturbances in patients with type 2 diabetes mellitus? J Clin Lab Anal 2018; 32: e22283.

5. Klisic A, Kavaric N, Jovanovic M, et al. Association between unfavorable lipid profile and glycemic control in patients with type 2 diabetes mellitus. J Res Med Sci 2017; 22: 122.

6. Čolak E, Pap D. The role of oxidative stress in the development of obesity and obesity-related metabolic disorders. J Med Biochem 2021; 40: 1-9.

7. García-Sánchez A, Miranda-Díaz AG, Cardona-Muñoz EG. The role of oxidative stress in physiopathology and pharmacological treatment with pro- and antioxidant properties in chronic diseases. Oxid Med Cell Longev 2020; 23: 2020:2082145.

8. Čolak E, Pap D, Nikolić Lj, Vicković S. The impact of obesity to antioxidant defense parameters in adolescents with increased cardiovascular risk. J Med Biochem 2020; 39: 346-54.

9. Klisic A, Kavaric N, Vujcic S, et al. Inverse association between serum endocan levels and small LDL and HDL particles in patients with type 2 diabetes mellitus. Eur Rev Med Pharmacol Sci 2020; 24: 8127-35.

10. Klisic A, Kavaric N, Ninic A. Retinol-binding protein 4 versus albuminuria as predictors of estimated GFR decline in patients with type 2 diabetes. J Res Med Sci 2018; 23: 44.

11. Rolle K, Piwecka M, Belter A, et al. The sequence and structure determine the function of mature human miRNAs. PLoS One 2016; 11: e0151246.

12. Jones DP, Sies H. The Redox Code. Antioxid Redox Signal 2015; 23: 734-46.

13. Sies H, Berndt C, Jones DP. Oxidative stress. Annu Rev Biochem 2017; 86: 715-48.

14. Halliwell B. The antioxidant paradox: less paradoxical now? Br J Clin Pharmacol 2012; 75: 637-44.

15. Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. Int J Physiol Pathophysiol Pharmacol 2019; 11: 45-63.

16. Yung JHM, Giacca A. Role of c-Jun N-terminal kinase (JNK) in obesity and type 2 diabetes. Cells 2020; 9: 706.

17. Zatterale F, Longo M, Naderi J, et al. Chronic adipose tissue inflammation linking obesity to insulin resistance and type 2 diabetes. Front Physiol 2020; 10: 1607.

18. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. Gastroenterology 2007; 132: 2169-80.

19. Nikolajczyk BS, Jagannathan-Bogdan M, Shin H, Gyurko R. State of the union between metabolism and the immune system in type 2 diabetes. Genes Immun 2011; 12: 239-50.

20. Tsalamandris S, Antonopoulos AS, Oikonomou E, et al. The role of inflammation in diabetes: current concepts and future perspectives. Eur Cardiol 2019; 14: 50-9.

21. Stefanović A, Kotur-Stevuljević J, Spasić S, Bogavac-Stanojević N, Bujisić N. The influence of obesity on the oxidative stress status and the concentration of leptin in type 2 diabetes mellitus patients. Diabetes Res Clin Pract 2008; 79: 156-63.

22. Stefanović A, Kotur-Stevuljević J, Spasić S, Vekic J, Bujisić N. Association of oxidative stress and paraoxonase status with PROCAM risk score. Clin Biochem 2009; 42: 617-23.

23. Stefanović A, Kotur-Stevuljević J, Spasić S, et al. HDL 2 Particles are associated with hyperglycaemia, lower PON1 activity and oxidative stress in type 2 diabetes mellitus patients. Clin Biochem 2010; 43: 1230-5.

24. Wu D, Wu C, Zhong Y. The association between paraoxonase 1 activity and the susceptibilities of diabetes mellitus, diabetic macroangiopathy and diabetic microangiopathy. J Cell Mol Med 2018; 22: 4283-91.

25. Pandey G, Shihabudeen MS, David HP, Thirumurugan E, Thirumurugan K. Association between hyperleptinemia and oxidative stress in obese diabetic subjects. J Diabetes Metab Disord 2015; 14: 24.

26. Klisic A, Isakovic A, Kocic G, et al. Relationship between oxidative stress, inflammation and dyslipidemia with fatty liver index in patients with type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 2018; 126: 371-8.

27. Klisic A, Kavaric N, Ninic A, Kotur-Stevuljevic J. Oxidative stress and cardiometabolic biomarkers in patients with non-alcoholic fatty liver disease. Sci Rep 2021; 11: 18455.

28. Klisic A, Kocic G, Kavaric N, Jovanovic M, Stanisic V, Ninic A. Xanthine oxidase and uric acid as independent predictors of albuminuria in patients with diabetes mellitus type 2. Clin Exp Med 2018; 18: 283-90.

29. Klisic A, Kavaric N, Stanisic V, et al. Endocan and a novel score for dyslipidemia, oxidative stress and inflammation (DOI score) are independently correlated with glycated hemoglobin (HbA1c) in patients with prediabetes and type 2 diabetes. Arch Med Sci 2020; 16: 42-50.

30. Klisic A, Kavaric N, Vujcic S, Spasojevic-Kalimanovska V, Kotur-Stevuljevic J, Ninic A. Factorial analysis of the cardiometabolic risk influence on redox status components in adult population. Oxid Med Cell Longev 2021; 6661940.

31. Kohata Y, Ohara M, Nagaike H, et al. Association of hemoglobin A1c, 1,5-anhydro-D-glucitol and glycated albumin with oxidative stress in type 2 diabetes mellitus patients: a cross-sectional study. Diabetes Ther 2020; 11: 655-65.

32. Sliwinska A, Kasinska MA, Drzewoski J. MicroRNAs and metabolic disorders – where are we heading? Arch Med Sci 2017; 13: 885-96.

33. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009; 19: 92-105.

34. Valencia-Sanchez MA, Liu J, Hannon GJ, Parker R. Control of translation and mRNA degradation by miRNAs and siRNAs. Genes Developm 2006; 20: 515-24.

35. Lim LP, Lau NC, Garrett-Engele P, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 2005; 433: 769-73.

36. Gregory RI, Yan KP, Amuthan G, et al. The Microprocessor complex mediates the genesis of microRNAs. Nature 2004; 432: 235-40.

37. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014; 15: 509-24.

38. Valinezhad Orang A, Safaralizadeh R, Kazemzadeh-Bavili M. Mechanisms of miRNA-mediated gene regulation from common downregulation to mRNA-specific upregulation. Int J Genomics 2014; 2014: 970607.

39. Borkowska EM, Kutwin P, Rolecka D, Konecki T, Borowiec M, Jabłonowski Z. Clinical value of microRNA-19a-3p and microRNA-99a-5p in bladder cancer. Arch Med Sci 2019 doi:10.5114/aoms.2019.89700.

40. Xie F, Liu J. Comprehensive analysis of the lncRNA-miRNA-mRNA ceRNA network in aortic dissection. Arch Med Sci 2020. doi:10.5114/aoms.2020.97354.

41. Zhang Q, Xiao X, Zheng J, et al. Vildagliptin, a dipeptidyl peptidase-4 inhibitor, attenuated endothelial dysfunction through miRNAs in diabetic rats. Arch Med Sci 2021; 17: 1378-87.

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42. Ardekani AM, Naeini MM. The role of microRNAs in human diseases. Avicenna J Med Biotechnol 2010; 2: 161-79.

43. Hashimoto N, Tanaka T. Role of miRNAs in the pathogenesis and susceptibility of diabetes mellitus. J Hum Genet 2017; 62: 141-50.

44. Poy MN, Hausser J, Trajkovski M, et al. miR-375 maintains normal pancreatic alpha- and beta-cell mass. Proc Natl Acad Sci USA 2009; 106: 5813-8.

45. Ou J, Kou L, Liang L, Tang C. MiR-375 attenuates injury of cerebral ischemia/reperfusion via targetting Ctgf. Biosci Rep 2017; 37: BSR20171242.

46. El Ouaamari A, Baroukh N, Martens GA, Lebrun P, Pipeleers D, van Obberghen E. miR-375 targets 3′-phosphoinositide-dependent protein kinase-1 and regulates glucose-induced biological responses in pancreatic beta-cells. Diabetes 2008; 57: 2708-17.

47. Hemmings BA, Restuccia DF. PI3K-PKB/Akt pathway. Cold Spring Harb Perspect Biol 2012; 4: a011189. Erratum in: Cold Spring Harb Perspect Biol 2015; 7. pii: a026609.

48. Norlin S, Ahlgren U, Edlund H. Nuclear factor-{kappa} B activity in {beta}-cells is required for glucose-stimulated insulin secretion. Diabetes 2005; 54: 125-32.

49. Gupta S, Sen S. Myotrophin-kappaB DNA interaction in the initiation process of cardiac hypertrophy. Biochim Biophys Acta 2002; 1589: 247-60.

50. Massart J, Sjögren RJ, Lundell LS, et al. Altered miR-29 expression in type 2 diabetes influences glucose and lipid metabolism in skeletal muscle. Diabetes 2017; 66: 1807-18.

51. Pullen TJ, da Silva Xavier G, Kelsey G, Rutter GA. miR-29a and miR-29b contribute to pancreatic beta-cell-specific silencing of monocarboxylate transporter 1 (Mct1). Mol Cell Biol 2011; 31: 3182-94.

52. Plaisance V, Abderrahmani A, PerretMenoud V, Jacquemin P, Lemaigre F, Regazzi R. MicroRNA-9 controls the expression of Granuphilin/Slp4 and the secretory response of insulin producing cells. J Biol Chem 2006; 281: 26932-42.

53. He A, Zhu L, Gupta N, Chang Y, Fang F. Overexpression of micro ribonucleic acid 29, highly up-regulated in diabetic rats, leads to insulin resistance in 3T3-L1 adipocytes. Mol Endocrinol 2007; 21: 2785-94.

54. Krek A, Grün D, Poy MN, et al. Combinatorial microRNA target predictions. Nature Genetics 2005; 37: 495-500.

55. Baroukh N, Ravier MA, Loder MK, et al. MicroRNA-124a regulates Foxa2 expression and intracellular signaling in pancreatic beta-cell lines. J Biol Chem 2007; 282: 19575-88.

56. Offield MF, Jetton TL, Labosky PA, et al. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development 1996; 122: 983-95.

57. Lovis P, Gattesco S, Regazzi R. Regulation of the expression of components of the exocytotic machinery of insulin-secreting cells by microRNAs. Biol Chem 2008; 389: 305-12.

58. Plaisance V, Abderrahmani A, Perret-Menoud V, Jacquemin P, Lemaigre F, Regazzi R. MicroRNA-9 controls the expression of Granuphilin/Slp4 and the secretory response of insulin-producing cells. J Biol Chem 2006; 281: 26932-42.

59. Ramachandran D, Roy U, Garg S, Ghosh S, Pathak S, Kolthur-Seetharam U. Sirt1 and mir-9 expression is regulated during glucose-stimulated insulin secretion in pancreatic beta-islets. FEBS J 2011; 278: 1167-74.

60. Chen Z, Shentu TP, Wen L, Johnson DA, Shyy JY. Regulation of SIRT1 by oxidative stress-responsive miRNAs and a systematic approach to identify its role in the endothelium. Antioxid Redox Signal 2013; 19: 1522-38.

61. Yu X, Liu Z, Fang J, Qi H. miR-96-5p: a potential diagnostic marker for gestational diabetes mellitus. Medicine 2021; 100: e25808.

62. Cai T, Long J, Wang H, Liu W, Zhang Y. Identification and characterization of miR-96, a potential biomarker of NSCLC, through bioinformatic analysis. Oncol Rep 2017; 38: 1213-23.

63. Yue C, Chen J, Li Z, Li L, Chen J, Guo Y. microRNA-96 promotes occurrence and progression of colorectal cancer via regulation of the AMPKα2-FTO-m6A/MYC axis. J Exp Clin Cancer Res 2020; 39: 240.

64. Carlomosti F, D'Agostino M, Beji S, et al. Oxidative stress-induced miR-200c disrupts the regulatory loop among SIRT1, FOXO1, and eNOS. Antioxid Redox Signal 2017; 27: 328-44.

65. Belgardt BF, Ahmed K, Spranger M, et al. The microRNA-200 family regulates pancreatic beta cell survival in type 2 diabetes. Nat Med 2015; 21: 619-27.

66. DA Silva ND Jr, Fernandes T, Soci UP, Monteiro AW, Phillips MI, DE Oliveira EM. Swimming training in rats increases cardiac MicroRNA-126 expression and angiogenesis. Med Sci Sports Exerc 2012; 44: 1453-62.

67. Ryu HS, Park SY, Ma D, Zhang J, Lee W. The induction of microRNA targeting IRS-1 is involved in the development of insulin resistance under conditions of mitochondrial dysfunction in hepatocytes. PLoS One 2011; 6: e17343.

68. Guo C, Sah JF, Beard L, Willson JK, Markowitz SD, Guda K. The noncoding RNA, miR-126, suppresses the growth of neoplastic cells by targeting phosphatidylinositol 3-kinase signaling and is frequently lost in colon cancers. Genes Chromosomes Cancer 2008; 47: 939-46.

69. Wu Y, Song LT, Li JS, Zhu DW, Jiang SY, Deng JY. MicroRNA-126 regulates inflammatory cytokine secretion in human gingival fibroblasts under high glucose via targeting tumor necrosis factor receptor associated factor 6. J Periodontol 2017; 88: e179-87.

70. Najafi-Shoushtari SH. MicroRNAs in cardiometabolic disease. Curr Atheroscler Rep 2011; 13: 202-7.

71. Davalos A, Goedeke L, Smibert P, et al. miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling. Proc Natl Acad Sci USA 2011; 108: 9232-7.

72. Rayner KJ, Suárez Y, Dávalos A, et al. MiR-33 contributes to the regulation of cholesterol homeostasis. Science 2010; 328: 1570-3.

73. Zhong L, D'Urso A, Toiber D, et al. The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1α. Cell 2010; 140: 280-93.

74. Kong L, Zhu J, Han W, et al. Significance of serum microRNAs in pre-diabetes and newly diagnosed type 2 diabetes: a clinical study. Acta Diabetol 2011; 48: 61-9.

75. Yang Z, Chen H, Si H, et al. Serum miR-23a, a potential biomarker for diagnosis of pre-diabetes and type 2 diabetes. Acta Diabetol 2014; 51: 823-31.

76. Zeinali F, Aghaei Zarch SM, Jahan-Mihan A, et al. Circulating microRNA-122, microRNA-126-3p and microRNA-146a are associated with inflammation in patients with pre-diabetes and type 2 diabetes mellitus: a case control study. PLoS One 2021; 16: e0251697.

77. Al-Muhtaresh HA, Al-Kafaji G. Evaluation of two-diabetes related microRNAs suitability as earlier blood bio-

markers for detecting prediabetes and type 2 diabetes mellitus. J Clin Forensic Med 2018; 7: 12.

78. Ursini F, Maiorino M, Forman HJ. Redox homeostasis: the golden mean of healthy living. Redox Biol 2016; 8: 205-15.

79. Leisegang MS, Schroder K, Brandes RP. Redox regulation and noncoding RNAs. Antioxid Redox Signal 2018; 29: 793-812.

80. Kalinina EV, Ivanova-Radkevich VI, Chernov NN. Role of MicroRNAs in the regulation of redox-dependent processes. Biochemistry 2019; 84: 1233-46.

81. Engedal N, Žerovnik E, Rudov A, et al. From oxidative stress damage to pathways, networks, and autophagy via microRNAs. Oxid Med Cell Longev 2018; 2018: 4968321.

82. Cheng X, Ku CH, Siow RC. Regulation of the Nrf2 antioxidant pathway by microRNAs: new players in micromanaging redox homeostasis. Free Radic Biol Med 2013; 64: 4-11.

83. Vezza T, de Marañón AM, Canet F, et al. MicroRNAs and oxidative stress: an intriguing crosstalk to be exploited in the management of type 2 diabetes. Antioxidants 2021; 10: 802.

84. LaPierre MP, Stoffel M. MicroRNAs as stress regulators in pancreatic beta cells and diabetes. Mol Metab 2017; 6: 1010-23.

85. La Sala L, Mrakic-Sposta S, Tagliabue E, et al. Circulating microRNA-21 is an early predictor of ROS-mediated damage in subjects with high risk of developing diabetes and in drug-naïve T2D. Cardiovasc Diabetol 2019; 18: 18.

86. Sun L, Li X, Li G, Dai B, Tan W. Actinidia chinensis Planch. Improves the indices of antioxidant and anti-inflammation status of type 2 diabetes mellitus by activating Keap1 and Nrf2 via the upregulation of microRNA-424. Oxid Med Cell Longev 2017; 2017: 7038789.

87. Yaribeygi H, Farrokhi FR, Butler AE, Sahebkar A. Insulin resistance: review of the underlying molecular mechanisms. J Cell Physiol 2019; 234: 8152-61.

88. Nathan DM. Long-term complications of diabetes mellitus. N Engl J Med 1993; 328: 1676-85.

89. Simeoli R, Fierabracci A. Insights into the role of microRNAs in the onset and development of diabetic neuropathy. Int J Mol Sci 2019; 20: 4627.

90. Mather KJ, Bebu I, Baker C, et al. Prevalence of microvascular and macrovascular disease in the Glycemia Reduction Approaches in Diabetes – A Comparative Effectiveness (GRADE) Study cohort. Diabetes Res Clin Pract 2020; 165: 108235.

91. Hadi HA, Suwaidi JA. Endothelial dysfunction in diabetes mellitus. Vasc Health Risk Manag 2007; 3: 853-76.

92. Tonyan ZN, Nasykhova YA, Danilova MM, Glotov AS. Genetics of macrovascular complications in type 2 diabetes. World J Diabetes 2021; 12: 1200-19.

93. Zhang JY, Gong YL, Li CJ, Qi Q, Zhang QM, Yu DM. Circulating MiRNA biomarkers serve as a fingerprint for diabetic atherosclerosis. Am J Transl Res 2016; 8: 2650-8.

94. Duan X, Zhan Q, Song B, et al. Detection of platelet microRNA expression in patients with diabetes mellitus with or without ischemic stroke. J Diabetes Complications 2014; 28: 705-10.

95. Olivieri F, Spazzafumo L, Bonafè M, et al. MiR-21-5p and miR-126a-3p levels in plasma and circulating angiogenic cells: relationship with type 2 diabetes complications. Oncotarget 2015; 6: 35372-82.

96. Zhong X, Chung AC, Chen HY, et al. miR-21 is a key therapeutic target for renal injury in a mouse model of type 2 diabetes. Diabetologia 2013; 56: 663-74.

97. Amr KS, Abdelmawgoud H, Ali ZY, Shehata S, Raslan HM. Potential value of circulating microRNA-126 and microRNA-210 as biomarkers for type 2 diabetes with coronary artery disease. Br J Biomed Sci 2018; 75: 82-7.

98. Al-Kafaji G, Al-Mahroos G, Abdulla Al-Muhtaresh H, Sabry MA, Abdul Razzak R, Salem AH. Circulating endothelium-enriched microRNA-126 as a potential biomarker for coronary artery disease in type 2 diabetes mellitus patients. Biomarkers 2017; 22: 268-78.

99. Jansen F, Yang X, Franklin BS, et al. High glucose condition increases NADPH oxidase activity in endothelial microparticles that promote vascular inflammation. Cardiovasc Res 2013; 98: 94-106.

100. Jansen F, Yang X, Baumann K, et al. Endothelial microparticles reduce ICAM-1 expression in a microRNA-222-dependent mechanism. J Cell Mol Med 2015; 19: 2202-14.

101. He X, Kuang G, Wu Y, Ou C. Emerging roles of exosomal miRNAs in diabetes mellitus. Clin Transl Med 2021; 11: e468.

102. Barutta F, Bellini S, Mastrocola R, Bruno G, Gruden G. MicroRNA and microvascular complications of diabetes. Int J Endocrinol 2018; 2018: 6890501.

103. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet 2010; 376: 124-36.

104. Yousefzadeh N, Alipour MR, Soufi FG. Deregulation of NF-κB-miR-146a negative feedback loop may be involved in the pathogenesis of diabetic neuropathy. J Physiol Biochem 2015; 71: 51-8.

105. Huang Y, Liu Y, Li L, et al. Involvement of inflammation-related miR-155 and miR-146a in diabetic nephropathy: implications for glomerular endothelial injury. BMC Nephrology 2014; 15: 142.

106. Bhatt K, Lanting LL, Jia Y, et al. Anti-inflammatory role of MicroRNA-146a in the pathogenesis of diabetic nephropathy. J Am Soc Nephrol 2016; 27: 2277-88.

107. Feng B, Chen S, McArthur K, et al. miR-146a-mediated extracellular matrix protein production in chronic diabetes complications. Diabetes 2011; 60: 2975-84.

108. Liu XS, Fan B, Szalad A, et al. MicroRNA-146a mimics reduce the peripheral neuropathy in type 2 diabetic mice. Diabetes 2017; 66: 3111-21.

109. Wang L, Chopp M, Szalad A, et al. The role of miR-146a in dorsal root ganglia neurons of experimental diabetic peripheral neuropathy. Neuroscience 2014; 259: 155-63.

110. Lee HW, Khan SQ, Khaliqdina S, et al. Absence of miR-146a in podocytes increases risk of diabetic glomerulopathy via up-regulation of ErbB4 and Notch-1. J Biol Chem 2017; 292: 732-47.

111. Kovacs B, Lumayag S, Cowan C, Xu S. MicroRNAs in early diabetic retinopathy in streptozotocin-induced diabetic rats. Invest Ophthalmol Vis Sci 2011; 52: 4402-9.

112. Chen Q, Qiu F, Zhou K, et al. Pathogenic role of microRNA-21 in diabetic retinopathy through downregulation of PPARα. Diabetes 2017; 66: 1671-82.

113. Lai JY, Luo J, O'Connor C, et al. MicroRNA-21 in glomerular injury. J Am Soc Nephrol 2015; 26: 805-16.

114. Dey N, Das F, Mariappan MM, et al. MicroRNA-21 orchestrates high glucose-induced signals to TOR complex 1, resulting in renal cell pathology in diabetes. J Biol Chem 2011; 286: 25586-603.

115. Zhong X, Chung AC, Chen HY, et al. miR-21 is a key therapeutic target for renal injury in a mouse model of type 2 diabetes. Diabetologia 2013; 56: 663-74.

116. Wang J, Duan L, Tian L, et al. Serum miR-21 may be a potential diagnostic biomarker for diabetic nephropathy. Exp Clin Endocrinol Diabetes 2016; 124: 417-23.

117. Fang S, Ma X, Guo S, Lu J. MicroRNA-126 inhibits cell viability and invasion in a diabetic retinopathy model via targeting IRS-1. Oncol Lett 2017; 14: 4311-8.

118. Liu R, Liu CM, Cui LL, Zhou L, Li N, Wei XD. Expression and significance of MiR-126 and VEGF in proliferative diabetic retinopathy. Eur Rev Med Pharmacol Sci 2019; 23: 6387-93.

119. Zheng Y, Liu Y, Wang L, et al. MicroRNA-126 suppresses the proliferation and migration of endothelial cells in experimental diabetic retinopathy by targeting polo-like kinase 4. Int J Mol Med 2021; 47: 151-60.

120. Greco M, Chiefari E, Accattato F, et al. MicroRNA-1281 as a novel circulating biomarker in patients with diabetic retinopathy. Front Endocrinol 2020; 11: 528.

121. Krupa A, Jenkins R, Luo DD, Lewis A, Phillips A, Fraser D. Loss of MicroRNA-192 promotes fibrogenesis in diabetic nephropathy. J Am Soc Nephrol 2010; 21: 438-47.

122. Kato M, Zhang J, Wang M, et al. MicroRNA-192 in diabetic kidney glomeruli and its function in TGF-beta-induced collagen expression via inhibition of E-box repressors. Proc Natl Acad Sci USA 2017; 104: 3432-7.

123. Kim S, Choi M, Cho KH. Identifying the target mRNAs of microRNAs in colorectal cancer. Comput Biol Chem 2009; 33: 94-9.

124. Fan B, Luk AOY, Chan JCN, Ma RCW. MicroRNA and diabetic complications: a clinical perspective. Antioxid Redox Signal 2018; 29: 1041-63.

125. Guo J, Li J, Zhao J, et al. MiRNA-29c regulates the expression of inflammatory cytokines in diabetic nephropathy by targeting tristetraprolin. Sci Rep 2017; 7: 2314.

126. Wilson N, Wright D. Inflammatory mediators in diabetic neuropathy. J Diabetes Metab 2011; S5: 4.

127. Li Y, Ma W, Xie C, et al. Identification of genes and signaling pathways associated with diabetic neuropathy using a weighted correlation network analysis: a consort study. Medicine 2016; 95: e5443.

128. Santos-Bezerra DP, Santos AS, Guimarães GC, et al. Micro-RNAs 518d-3p and 618 are upregulated in individuals with type 1 diabetes with multiple microvascular complications. Front Endocrinol 2019; 10: 385.

129. Chen X, Yu X, Li X, et al. MiR-126 targets IL-17A to enhance proliferation and inhibit apoptosis in high-glucose-induced human retinal endothelial cells. Biochem Cell Biol 2020; 98: 277-83.

130. Qin LL, An MX, Liu YL, Xu HC, Lu ZQ. MicroRNA-126: a promising novel biomarker in peripheral blood for diabetic retinopathy. Int J Ophthalmol 2017; 10: 530-4.

131. Park S, Moon S, Lee K, Park IB, Lee DH, Nam S. Urinary and blood microRNA-126 and -770 are potential non-invasive biomarker candidates for diabetic nephropathy: a meta-analysis. Cell Physiol Biochem 2018; 46: 1331-40.