

Focus on Diabetic Retinopathy (DR) and MicroRNA “What Association”

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ABSTRACT

Diabetes Mellitus (DM) is one of the most common chronic diseases in the world. According to the latest data from the International Diabetes Federation, it is estimated that around 700 million will develop DM by 2045!

Glycemic control is crucial in the management of DM and for the prevention of microvascular “nephropathy, retinopathy, neuropathy” and macrovascular complications. Of all the microvascular complications, the one that aroused most interest in our descriptive study was Diabetic Retinopathy (DR), since, if left untreated, it leads to irreversible anatomical and functional damage. DR is the leading cause of blindness in working-age adults across the world. The pathogenesis of DR is multifactorial and the molecular mechanisms are still not fully understood. The treatment of DR essentially involves laser therapy, which is not always decisive due to absence and/or limited availability of successful drug therapies for these blinding disorders. The clinical diagnosis of DM often occurs late and this delay means that the chronic vascular complications associated with the disease are already present at the time of diagnosis. Therefore, this risk can be reduced if an early diagnosis is made. Hence the need to identify new specific biomarkers that can act as diagnostic and prognostic indicators of the disease.

According to some studies, putative roles of small endogenous molecules of microRNAs as indicators of DR and associated complications are being considered. They carry out their silencing activity on a wide range of transcripts deriving from the expression of thousands of genes.

There is evidence for the aberrant expression of microRNAs implicated in the onset of numerous pathologies. Since most of the genetic variations associated with diabetes are found in regions of the human genome that are not coding for proteins, it is worth studying those microRNAs involved in DR, both as possible therapeutic targets and as specific predictive diagnostic biomarkers. The present study aims to highlight the importance of some microRNAs and indicate those most implicated in the onset of DR, according to the literature, for the purpose of making a previous diagnosis of diabetes, in particular of DR.

Keywords: Diabetic Retinopathy, MicroRNA, Diabetes, Retinopathies

Introduction

The term “Retinopathies” refers to a large group of pathologies affecting the retina and greater attention should be paid as they can cause loss of vision. In terms of incidence, in our society, the main diseases affecting the retina concern: age-related macular degeneration, pigmentary retinopathy, macular edema and DR (Mayur Choudhary and Goldis Malek., 2016).

The retinopathies are characterized by an alteration of the ocular vascular component, with greater expression of different factors by retinal endothelial cells including the endothelial vascular growth factor (Jack Kanski and Brad Bowling, 2015). In fact, VEGF is responsible for a series of pro angiogenic effects characterized by vasodilation, by the increase in the permeability of blood vessels, by the release of proteolytic enzymes that invade and condition tissue remodeling with prevention of endothelial apoptosis (Yun-Zheng, 2017). Diabetes mellitus is an important cardiovascular risk factor characterized by elevated plasma glucose levels.

High glucose (HG) negatively influences endothelial cell (EC) function, which is characterized by the inability of ECs to respond to vascular endothelial growth factor (VEGF-A) stimulation (Masuda *et al.*, 2017).

DR is a chronic, progressive disease, potentially dangerous for retinal microvascularization, associated with pathophysiological changes intensified by diabetes. DR is the most frequent and the most important ocular complication of Diabetes Mellitus (DM), characterized by more or less extensive damage to the retina and represents, according to data from the World Health Organization (WHO), one of the main causes of low vision/blindness in adults (Das and McGuire, 2003). The prevalence of DR is directly related to the duration of diabetes: after twenty years of diabetic disease, almost all patients with DMT1 and more than 70% of patients with DMT2 develop a certain degree of DR (Lee *et al.*, 2015; Yau *et al.*, 2012). As the global prevalence of diabetes increases, the incidence of DR also increases. Approximately one-third of individuals with diabetes will develop some degree of retinopathy, which currently is the leading cause of blindness in patients aged 20-74 years, in the developed and developing countries (Cheloni *et al.*, 2019; Ruta *et al.*, 2013).

The Italian epidemiological data available, although not representing the reality of the country, show that at least 30% of diabetics have retinopathy and that 1% is affected each year by the most severe forms of the same (Italian standards for the treatment of diabetes mellitus, 2018).

Mechanisms Underlying Diabetic Retinopathy

The microvascular circulation of the retina is predominantly affected by DM, resulting in a range of structural changes. These changes ultimately cause increased permeability and altered proliferation of endothelial cells (ECs), edema, and abnormal neovascularization (NV) of the retina, leading to vision loss (Qiaoyun Gong and Guanfang Su, 2017). The pathogenesis of DR is extremely complicated, implicating numerous different mechanisms. Like other parts of the nervous system, the retina requires a continuous supply of oxygen.

If the circulation is completely cut off by a sudden increase of intraocular pressure (IOP) to levels above systemic arterial pressure, vision in humans is altered (Linsenmeier and Zhang, 2017).

DR causes a state of suffering in the retinal bloodstream that degenerates into a condition of retinal hypoxia (slowing down and reduced supply of oxygen to the retina). As in other body areas, when a tissue no longer receives blood and therefore oxygen, it degenerates. At the retinal level, the blood vessels become weaker, change their morphology, give rise to repeated edema and bleeding that in the long term alter the retinal tissue. This stage of retinopathy is defined as not proliferating. If these ischemic alterations are accentuated or long lasting, a process can be established whereby abnormal blood vessels form in the retina to suppress the lack of oxygen; however, the structure of these new abnormal vessels is very weak and therefore they undergo repeated bleeding processes (Cheung *et al.*, 2010).

These abnormal blood vessels or new blood vessels proliferate and destroy the retinal tissue giving rise to fibrotic (cicatricial) processes with possible retinal breakage and detachment (proliferating retinopathy) (Sorrentino *et al.*, 2018).

DR is based on vascular damage, caused mainly by biochemical alterations related to hyperglycemia with a consequent increase in vascular permeability, and by a high state of tissue hypoxia with overexpression of VEGF, inflammatory cytokines and oxygen reactive species (Cohen and Gardner, 2016).

Reactive oxygen species are reactive intermediates of molecular oxygen that act as important second messengers within the cells; however, an imbalance between generation of reactive ROS and antioxidant defense systems (Fig. 1) represents the primary cause of endothelial dysfunction, leading to vascular damage in both metabolic and atherosclerotic diseases (Anna Votano *et al.*, 2021; Incalza *et al.*, 2018). Furthermore, new mediators of vascular damage, such as microRNAs, will be discussed.

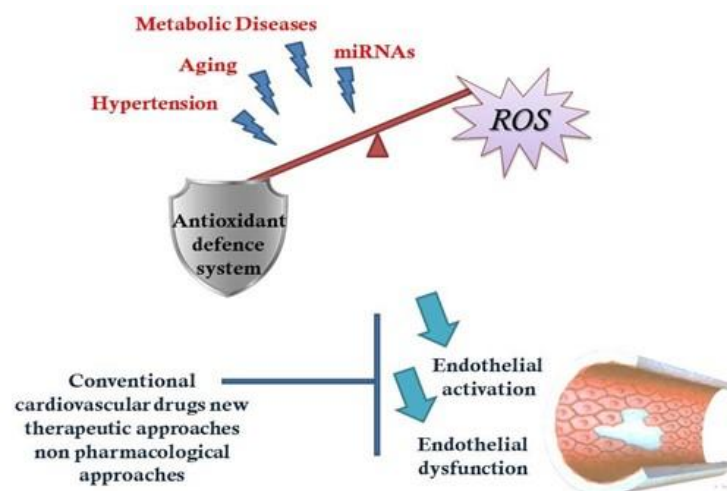


Figure 1: Reactive ROS and antioxidant defense systems: primary cause of endothelial dysfunction

The development and progression of complications depend on the interaction between the genes, epigenetic changes due to the environment, insulin resistance, immune dysregulation and inflammation (Fig. 2). Diabetes and its complications result from common pathophysiologicals.

Alteration of inflammatory responses usually occurs in the early stage of DR, which upregulates the expression of inflammatory markers, such as intercellular adhesion molecule 1 (ICAM1) and tumor necrosis factor- α (TNF- α). Microangiopathy is due to an alteration of the capillaries, with capillary permeability, hypertrophy of the capillary cells, increase in endothelial microvilli. DR consists of a microangiopathy where small vessels are particularly sensitive to damage caused by high glucose levels. In the pathogenesis of DR hyperglycaemia is the causative factor, causing a series of reactions that include the increase in the production of catabolic products of glycosylation, the activation of the metabolism of the polyol pathway and the altered transduction of cellular signals (Yao *et al.*, 2019). The damage to the pericytes, mesenchymal cells with contractile function that partially surrounds the endothelial cells, results in an imbalance in the mechanisms of regulation of flow controls with consequent hypoxia and accumulation of fluid inside the retinal tissue (Sinclair and Schwartz, 2019) as reported in this recent study.

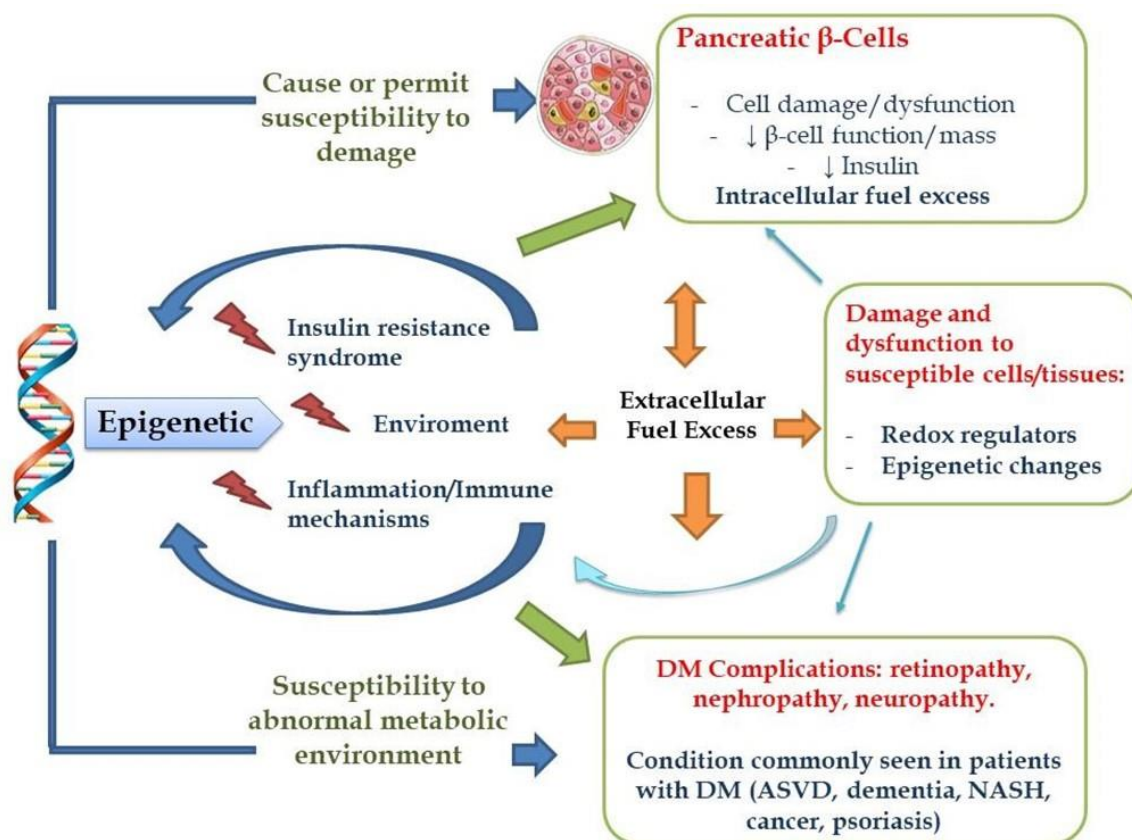


Figure 2: The development and progression of complications depend on the interaction between the genes, epigenetic changes due to the environment, insulin resistance, immune dysregulation and inflammation

The treatment of DR essentially involves laser therapy, which is not always decisive due to absence and/or limited availability of successful drug therapies for these blinding disorders. Currently, the main treatment for retinal neovascularization involves intravitreal injection of anti-VEGF agents, such as ranibizumab and bevacizumab (America Diabetes Association, 2013).

However, some patients show poor or no response to anti-VEGF agents with limited or no visual improvement (Kruger *et al.*, 2013), suggesting that other molecules “e.g., PDGF and basic FGF” may be involved in retinal neovascularization (Abramsson *et al.*, 2003).

In association with this treatment, the patient should observe adequate daily physical activity, to maintain good metabolic control, a personalized diet associated with any oral hypoglycaemic agents or insulin and drug therapy (Frank, 1997).

Genes, Environmental Factors, Epigenetic Changes

Oxidative stress induced by hyperglycaemia and glucolipototoxicity determines changes in chromatin structure that mediate changes in gene expression with consequent upregulation of proinflammatory and profibrotic mediators (Goyal *et al.*, 2019).

These epigenetic changes are recognized as a key factor in the development and progression of diabetic vascular complications. Epigenetic modifications are generally produced by external changes (toxins, nutrients, etc.) that transduce inside cells to cause DNA methylation and histone modification which leads to an alteration of gene expression (Rodriguez and El-Osta, 2018).

In contrast with genetic modifications, epigenetic changes are susceptible to modifications by the environment as well as dysregulation over time; DNA methylation, histone modification, and non coding RNA are three major types of epigenetic effects (Feng *et al.*, 2016; Ceriello *et al.*, 2014). We are not aware of studies specifically examining epigenetic modulation of endothelial function in diabetes. Obviously larger scale and prospective investigations are needed to establish whether epigenetic changes affect the risk of developing diabetes in general, and endothelial dysfunction in particular (Muka *et al.*, 2016). Altered gene expression following epigenetic changes leads to cell hypertrophy, proliferation, remodeling and apoptotic signaling and are key factors in the development and progression of diabetic complications. In fact, the expression of many genes implicated in the metabolic pathways associated with the development of diabetic retinopathy has been observed and have been increased or downgraded and changes have also been observed in the expression of microRNAs that play key roles in diabetic retinopathy, including rupture of the blood retina and the expression of neovascularization (Giovanni Faglia *et al.*, 2013).

It is estimated that more than 60% of protein coding genes in humans are regulated by miRNAs (Friedman *et al.*, 2009). MicroRNAs, a class of non-coding RNAs, are important regulators of gene expression that exert their function through post-transcriptional silencing of complementary mRNA targets.

MicroRNA and DR

MicroRNAs modulate biologic processes at the post-transcriptional level by binding to the 3'-untranslated region (3'-UTR) of their target genes, leading to translational repression or degradation (Tang *et al.*, 2020). Diabetic patients exhibit altered expression profiles of miRNAs involved in angiogenesis, vascular repair, and endothelial homeostasis. Of these, miRNA126 is strongly linked with vascular repair. Thus, patients with type 2 diabetes (T2DM) exhibit low levels of circulating miRNA126 compared with healthy subjects (Yi Shi and Paul, 2017).

According to recent transcriptome analyses, certain miRNA species are expressed in all retinal cell types, while others are cell type-specific. As miRNAs play important roles in homeostasis, cellular function, and survival of differentiated retinal cell types, their dysregulation is associated with retinal degenerative diseases (Zuzic *et al.*, 2019).

In recent years, miRNAs have been shown to be involved in various biologic processes, including proliferation, differentiation, development, and metabolism, as well as in various diseases.

Some 250 miRNAs are expressed in the retina where they are components of a complex gene regulatory network (Karali *et al.*, 2010; Krol *et al.*, 2010; Sundermeier and Palczewski, 2012). Key processes regulated by miRNAs in the retina include adaptation to different light intensities, rapid turnover of the phototransduction cascade and maintenance of cellular homeostasis under high activity. Furthermore, miRNAs are implicated in control of cell differentiation, maturation and survival in retina (Chung *et al.*, 2015).

Abnormal development of blood vessels within the retina plays important roles in many ocular neovascular diseases, including retinopathy of prematurity, proliferative diabetic retinopathy, and wet age-related macular degeneration. Endothelial cell proliferation and migration lead to the angiogenic growth of new blood vessels sprouting from retinal veins, and may result in vitreous hemorrhage, retinal detachment, and even blindness (Bharadwaj *et al.*, 2013). Previous studies have shown that retinal angiogenesis is regulated by many angiogenesis-related factors, including vascular endothelial growth factor platelet-derived growth factor (PDGF), fibroblast growth factor [FGF], and transforming growth factor (Yang *et al.*, 2016).

MicroRNA and Inhibition of Endothelial Cell Proliferation in DR

Just like elsewhere in the body, the function of miRNAs has shown increasing relevance in the eye. In the back of the eye, the retina consists of a thin layer of neuronal tissue of diverse cell types and is equipped with a highly specialized light-sensing capacity (Liu *et al.*, 2020) (Fig. 3). Fine-tuning gene expression for cell differentiation and function is crucial for vertebrate retinal development and proper vision.

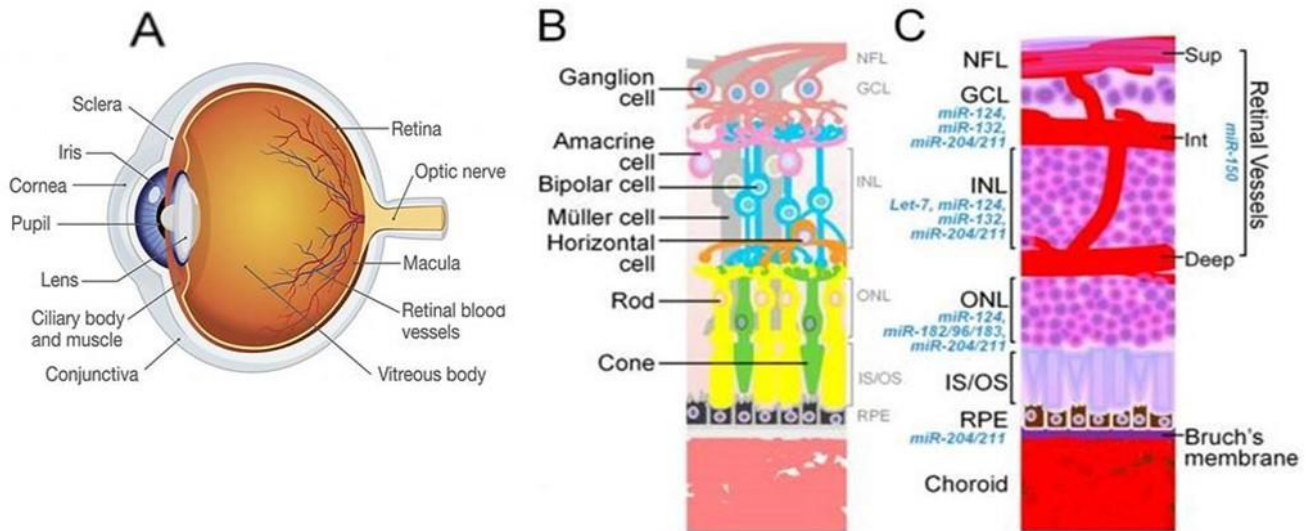


Figure 3: The Anatomy of The Eye and Relevant miRNAs

(A) The schematic diagram illustrates the main structures of the human eye. (B) The schematic representation of the cell types in the neural retina depicts their cellular connections (including ganglion cells, amacrine cells, bipolar cells, horizontal cells, as well as rod and cone photoreceptors) and supporting cells (Müller cells and RPE). (C) A cross section of the eye shows the laminar organization of the nuclear layers (GCL, INL, and ONL), the retinal vasculature, and segments of photoreceptors (IS/OS). The RPE monolayer, with Bruch's membrane underneath, is located between the neural retina and the choroid complex. miRNAs that regulate the physiological functions or pathological conditions related to each retinal neuronal and vessel layers, and RPE, are listed next to respective histological structure.

Abnormal angiogenesis disrupts the delivery of oxygen and nutrients, which can lead to an unbalanced metabolic status, and result in structural instability and functional loss of affected tissues. Excessive angiogenesis, also known as neovascularization, has considerable pathophysiologic roles in DR (Yang *et al.*, 2020).

In some studies, several miRNAs had reported important roles in DR (Liu *et al.*, 2018; Zhang *et al.*, 2017).

For example, miR-15a has been shown to regulate angiogenesis in DR through targeting VEGF. MiR-451 inhibited the proliferation and migration of retinal pigment epithelial cells in proliferative DR (Li X and e Shao, 2015). MiR-200b and miR-29a have also been involved in the development of DR. MicroRNA200b is downregulated in retinal endothelial cells and cardiac microvascular endothelial cells exposed to high glucose, and its overexpression reduces VEGF expression and prevents diabetic retinopathy and cardiomyopathy (McArthur *et al.*, 2011).

Accumulated evidence has revealed that miRNAs play important roles in endothelial cell dysfunction and angiogenesis. In this study it was seen that the miR-29B-3B inhibits the proliferation and angiogenesis of retinal microvascular endothelial cells (RMEC), while inhibition of miR-29b-3p had opposite effects. These effects of miR-29b-3p on RMEC were mediated by the negative regulation of the expression of VEGFA and PDGFB (Tang *et al.*, 2020).

Recent study has revealed that miR-29b regulates VEGF-A expression by binding directly to their 3'-UTR (Li P *et al.*, 2013). With this study, miR-29b-3p was found to regulate VEGF-A expression negatively, while inhibition of miR-29b-3p increased VEGF-A expression.

In this study (Chen *et al.*, 2019) has been studied that miR-144-3p significantly inhibited cell proliferation induced by high glucose (HG) through suppressing fibroblast growth factor (FGF)16, suggesting miR-144-3p as a potential target for DR therapy. In previous studies, it has been seen that miR-144-3p was down-regulated in rat DR model, which was further confirmed in the present study

Analysis of circulating miRNAs from serum or plasma samples of DM patients both with and without DR showed altered expression levels of many miRNAs throughout different patient populations (e.g., ages, type of DM, years after onset, etc.,) (Wu JH *et al.*, 2012).

The miRNA146 family participates in the regulation of oxidative stress and the production of proinflammatory factors. Circulating levels of miR146a are decreased in diabetic patients. Among them, miR-27b and miR-320a are the two miRNAs mostly associated with the risks of DR in T1DM (Qing *et al.*, 2014). MiR-221, an anti-angiogenic miRNA, is significantly altered in DM and is involved in the DM physiopathology and macrovascular complications associated with T2DM (Zampetaki *et al.*, 2016; Liu HN *et al.*, 2018; Fiorentino *et al.*, 2013; Costantino *et al.*, 2016). In addition, other circulating miRNAs including miR-126 (Lightell Jr *et al.*, 2018), miR-150 (Qin LL *et al.*, 2017), miR-155 (Mazzeo *et al.*, 2018), and miR-200b (Yang TT *et al.*, 2015) are also dysregulated in DR patients, as well as in preclinical animal models of DR (Li EH *et al.*, 2017; Ye P *et al.*, 2014; Bai Y *et al.*, 2011; Wang and Yan, 2016; McAuley *et al.*, 2015).

These findings indicate the complex regulation of miRNAs in DR and the potential of miRNAs as biomarkers and/or therapeutic targets for treating DR (Qing *et al.*, 2014).

These results indicated significant roles of miRNA in DR and consequently we wanted to indicate the main microRNAs involved in DR.

Conclusions

A systematic search of English-language articles in PubMed database was performed using the search terms of the headings of medical subjects [MeSH] “diabetic retinopathy”, “vascular endothelial growth factor”, “VEGF”, “ADMA”, “microRNA and diabetic retinopathy”, “diabetic retinopathy and endothelial dysfunction”.

The research date range was limited to a period between February 2012 and January 2020, except for 7 articles. Only articles and/or experimental studies that analyzed and studied microRNAs were included; such as: miR-15a, miR-451, miR-200b, miR-29a, miR-29b-3p, miR-144-3p, miR-27b, miR-320a, miR-221, miR-150, miR-155.

During our research, only diabetic retinopathy was included as a microvascular complication of DM. Clinical cases and “gray literature” articles have been excluded.

Since the discovery of the first microRNA (miRNA) decades ago, miRNA biology studies have expanded into many fields of biomedical research, including ocular research. Although microRNAs (miRNAs) provide a recently recognized level of regulation of gene expression, the retinal miRNA transcriptome and the contribution of miRNAs to retinal development and function are largely unknown.

Therefore, in addition to diagnosing DR through histological examination of the fundus vasculature, circulating miRNAs were found to play critical roles in DR development, suggesting that miRNAs could also serve as new biomarkers in detecting or predicting the progress of retinopathy and furthermore, the overall progress of DM. Analysis of circulating miRNAs from serum or plasma samples of DM patients both with and without DR showed altered expression levels of many miRNAs throughout different patient populations, such as: ages, type of DM, years after onset, etc.

MiRNA-targeted treatments, particularly those that interfere with the balance between VEGF and NO and endothelial progenitor cell function, may be promising avenues for therapeutically addressing endothelial dysfunction in diabetes. This hypothesis requires further experiments to validate and more clinical studies to be verified. In conclusion, this study adds to the growing number of tests involving miRNA in regulating angiogenesis. To begin to understand the functions of miRNAs in the retina, we

wanted to indicate just some of the many microRNAs involved in DR. There are several ongoing clinical studies designed to test the efficacy of miRNAs in the treatment of various disorders, so that gene therapy miRNA-based may become a new therapeutic option for a wide range of diseases in the future.

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