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The role of micro-RNA (miRNA) 126 on diabetic macular edema. Literature review

El papel del micro-ARN (miARN) 126 en el edema macular diabético. Revisión de literatura

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SUMMARY

Diabetic macular edema (DME), a further complication of diabetic retinopathy, is a clinical manifestation of a breakdown of the blood-retinal barrier in the form of thickening of the retinal fovea, exudates formation on the retina, and cystoid macular edema. The breakdown mechanism is based on the process of unstabilized vascular integrity, angiogenesis, and inflammation. A combination of these mechanisms, among others, is well known to be implemented by VEGF. Micro RNA (miRNA / miR) with its pleiotropic nature is an attractive alternative strategic therapy, especially in diseases with a multifactorial origin and its effective therapy has not been found. Micro RNA 126 which has a variety of pleiotropically targeted genes, cell, and organ functions, and approaches the retinal barrier breakdown mechanism, acts as an attractive candidate for initiation and completion of future trials.

Keywords: *Micro RNA, diabetic macular edema, retina.*

RESUMEN

El edema macular diabético (EMD), una complicación adicional de la retinopatía diabética, es una manifestación clínica de una ruptura de la barrera hematorretiniana en forma de engrosamiento de la fóvea retiniana, formación de exudados en la retina y edema macular cistoide. El mecanismo de descomposición se basa en el proceso de integridad vascular no estabilizada, angiogénesis e inflamación. Se sabe que la VEGF implementa una combinación de estos mecanismos, entre otros. El micro ARN (miARN/miR) con su naturaleza pleiotrópica

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constituye una terapia estratégica alternativa atractiva, especialmente en enfermedades con origen multifactorial y no se ha encontrado su terapia efectiva. El microRNA 126, que tiene una variedad de genes dirigidos pleiotrópicamente, funciones de células y órganos, que se acerca al mecanismo de ruptura de la barrera retiniana, actúa como un candidato atractivo para el inicio y finalización de ensayos futuros.

Palabras clave: *Micro ARN, Diabetic macular edema, retina*

1. INTRODUCTION

Diabetic retinopathy (DR) is one of the main causes of blindness in the working age and elderly population. Almost all of type 1 DM (T1DM) and more than 60 % (84 % with insulin and 53 % without insulin) of those with type 2 DM (T2DM) get "varying" degrees of diabetic retinopathy after 20 years of diabetes (1).

Diabetic Macular Edema (DME), an advanced complication of DR, can affect up to 7 % of people with diabetes mellitus (2,3). The initial stage of DR is non-proliferative diabetic retinopathy (NPDR) characterized by dot blot bleeding and vascular abnormalities such as microaneurysms and permeability that can cause DME. In the advanced stages, namely proliferative diabetic retinopathy (PDR), retinal hypoxia occurs which results in the growth of new blood vessels or neovascularization, vitreous bleeding, the proliferation of pre-retinal fibrovascular, and tractional retinal detachment. Sharp vision is not always affected at an early stage, but further disease development can cause significant vision loss (4).

In patients with T2DM, DME is the most common cause of vision loss. It has been reported that DME affects around 75,000 new patients in the United States each year. A review of the literature on disease burden revealed that patients with DME consume significantly more health resources and incur higher costs compared to patients with diabetes without retinal complications (5,6).

The role of genetics in DR risk factors is considered because from several observations it is found that controlling blood glucose levels, dyslipidaemia, and blood pressure alone is not enough to control the development of DR. In a cohort study it was also found that 42.6 % of T1DM sufferers for 50 years had not experienced PDR (7). Efforts to identify genes as part of risk factors include several studies including the Candidate Genes study; Linkage studies and Genome Wides Association (GWAS) studies (8). GWAS has facilitated a substantial and rapid rise in examining all the DNA variations, especially in SNPs (Single Nucleotide Polymorphism) in the Genome (9). Future clinical trials will likely continue the revolutionary findings from the Human Genome Project that RNA transcripts far outnumber protein-encoding genes. Classes of nonprotein-coding RNA (ncRNAs) have vastly expanded with ongoing research studies and now include transcript RNA such as microRNA (miRNAs), natural antisense transcript, Piwi-interacting RNA (piRNA), and long ncRNAs (lncRNAs).

MicroRNAs (miRNAs) themselves are endogenous non-coding RNAs, with a length of 20-22 nucleotides, which regulate gene expression at the post-translation level (10). A common characteristic of a miRNA is its ability to pleiotropically target, potentially hundreds or even thousands of genes, and some function in an organ or cell-specific manner. Correspondingly, this opens up the possibility of "one" miRNA candidate having the capability to regulate entire biological pathways that are pathogenically disrupted in a patient (11).

MicroRNA (miRNA) plays an important role in the pathological process of diabetic retinopathy, including the inflammatory response, insulin signaling, and angiogenesis. In addition to the regulatory function in gene expression, miRNA is considered a potential (protein) therapeutic target, as well as a diagnostic marker for many diseases. An understanding of the pathological mechanisms underlying diabetic retinopathy is still incomplete and additional investigations are needed to develop new therapeutic strategies (12).

Further new therapeutic options on miRNA occur, cause traditionally targeting single protein or nodes in biological pathways often failed to reverse pathogenic phenotypes, and sum small molecule drugs have successfully been pharmacologically designed to pleiotropically target multiple targets

or pathways, providing further support to miRNA as future therapeutic (11).

2. DIABETIC RETINOPATHY

Diabetic Retinopathy (DR) is a complex disease that can be divided into nonproliferative and proliferative. Proliferative Diabetic Retinopathy (PDR) is a condition of further disease where usually retinal nutrient circulation has failed and the tissue becomes oxygen-deficient or ischemic resulting in the formation of fragile and leaky neovascularization. Non-proliferative Diabetic Retinopathy (NPDR) is an early stage of the disease that can be asymptomatic or with very mild symptoms. In this situation, there is neuronal loss, local inflammation, and loss of pericyte which leads to the formation of microaneurisms that can result in leakage of fluid to the retina. When fluid leaks into the macular region, diabetic macular edema (DME) occurs. Diabetic macular edema can occur at any stage of diabetic retinopathy (13,14).

The pathogenesis of DR is very complicated, involving many mechanisms, including a consistent increase in blood glucose levels that will cause stress oxidase which is characterized by an increase in reactive oxygen species (ROS) interlinked with the advanced glycation end product (AGE) and Vascular Endothelial Growth Factors (VEGF) will contribute to increased inflammatory factors at an early stage, followed by the emergence of pro-inflammatory cytokines (15,16).

2.1 The Nature of Diabetic Retinopathy

Hyperglycemic conditions and metabolic changes from diabetes cause proliferative changes in blood vessels as a result of decreased quality of perfusion in retinal tissue. This relative state of ischemia in the retina is strongly suspected as a primary angiogenic stimulus that plays a central role in the pathogenesis of PDR (17).

The natural development of Proliferative Diabetic Retinopathy (PDR) is illustrated by the formation of "new pathological blood vessels" in the retina and optic disc that will develop on the surface of the retina and enter the vitreous (17). This new blood vessel grows progressively with the formation of surrounding fibrovascular tissue. The contraction of the fibrous tissue can cause tractional retinal detachment and vitreous haemorrhage, two of the most common complications causing blindness in PDR (17). Some angiogenic factors considered responsible for this process are angiopoietin, erythropoietin, basic Fibroblast Growth Factor (bFGF), Insulin-Like Growth Factor (IGF), Protein Kinase C (PKC), Platelet-Derived Growth Factor (PDGF). But based on in-vivo and in-vitro studies, it turns out that the VEGF (Vascular Endothelial Growth Factor) protein remains a major cause of these pathological changes in the retina triggered by ischemia (17). PDR sufferers, whether treated or not, will generally continue to develop to a certain stage (advanced) which will then remain like that for almost a decade. The remaining vision depends on the degree of damage to the structure of the retina at the last moment (17).

3. DIABETIC MACULAR EDEMA (DME) PATHOPHYSIOLOGY BASED ON BRB

High and uncontrolled blood glucose levels are the main cause of type 2 DM and related complications. DR is a chronic and serious eye complication associated with DM, causing microvascular and macrovascular changes. In the early stages of the DR, human retinal cells (HREC) and retinal pigment epithelial cells (RPE), which are components of the retinal-blood barrier (BRB), are affected and damaged by the adverse effects of high glucose (HG). This induces BRB dysfunction and contributes to the development of DR (18).

Diabetic macular edema (DME) is not a uniform condition and can be easily divided into different disease subtypes. However, a universal scoring system is still lacking for this condition. Therefore, DME raises many questions including why current treatments are not effective for all patients and why patient lifestyles do not always correlate with disease progression. To answer these questions, we need to better understand the complex and multifactorial pathophysiological mechanisms involved in the disease process (19).

Diabetic macular edema (DME) is thought to occur as a result of the impaired blood-retinal barrier (BRB), which causes swelling or

thickening of the macular area as a result of the accumulation of sub and intraretinal fluid on the inner and the outer plexiform layer (19,20,21) Several risk factors for the development of diabetic eye disease have been well identified and documented. This includes poor blood glucose control, high blood pressure, and high lipid levels. Hyperglycaemia from poor blood glucose control leads to a chain of damaging tissue responses in the retina that results in the formation of free radicals (oxidative damage), microthrombi formation (microscopic clusters of fibrin, platelets, and red blood cells), activation of cell adhesion molecules and activation of leukocytes and cytokines that produce overexpression mediated by ischemia (from growth factors and cytokines). However, damage from BRB is caused by changes in the permeability characteristics of retinal endothelial cells due to increased levels of various growth factors and cytokines which then result in subsequent vascular dysfunction. Inflammation likely plays a key role in some of the pathogenesis subtypes of DME, and the observation of phenotypes are very similar to cystoid macular edema (CME) edema seen in uveitis and Irvine-Gass syndrome (19,22-24). In addition to the many risk factors that have been proposed due to the complex metabolic environment of the retina, single nucleotide polymorphisms (SNPs) in various genes involved in this pathway have been shown to have effects on the growth and development of diabetic retinopathy in different populations. The group of genes studied are predominantly those with known metabolic or functional roles in diabetes (13,25).

DME is characterized by the presence of microaneurisms, exudates, and cystic-shaped intraretinal fluid. Based on its distribution in the macula, DME can provide a focal or diffuse picture. Based on the Early Treatment Diabetic Retinopathy Study (ETDRS), clinically significant macular edema is defined as (i) retinal thickening in 500 microns from the fovea center; (ii) exudate in 500 microns from the foveal center, and by thickening of the adjacent retina or (iii) thickening of the retina at least one optical disc diameter and in one optical disc diameter from the fovea center (26-29).

Eye organs have mechanisms that block the passage of certain substances or microorganisms to reduce the risk of inflammation. This mechanism involves several barriers: (i) Retinal Pigment Epithelium (RPE), which acts as an external Blood Retinal Barrier (e-BRB); (ii) Retinal vascular plexus, which acts as inner BRB (i-BRB); (iii) Pigmented capillary endothelial cells from the iris epithelium that form the anterior hematoakuos barrier and (iv) Epithelial ciliary processes without pigments, which form the posterior hematoakuos barrier (30,31).

3.1 Role of External Blood Retinal Barrier (e-BRB) And Retinal Pigment Epithelium (RPE)

RPE is an external retinal layer whose functions include: (i) Regeneration of all-trans-retinol to 11-cis-retinal which is an important process for vision, which supplies vitamin A and glucose to photoreceptors, (ii) Phagocytosis of external discs and old photoreceptors that are old undergoes degradation during the visual cycle, (iii) Nutritional functions which allow oxygen and nutrients to move from the choriocapillaris to the external segment of the retina, and (iv) Diffusion of molecules into RPE. RPE cells have tight junctions between cells that act as e-BRB, determining the exchange of substances controlled between the retinal neurosensory and choriocapillaris. RPE pumps fluid into choriocapillaris to prevent the formation of macular edema (30).

3.2 Inner-Blood Retinal Barrier (i-BRB)

The retinal capillary has two different plexuses namely the superficial capillary plexus is located in the ganglion cell layer (GCL) and the capillary plexus is located in the inner nuclear layer (INL), adjacent to the inner plexiform layer (IPL) and synaptic of the outer plexiform layer (OPL). Two retinal capillary plexus endothelial cells are strongly connected by tight junctions and adherent junctions, each of which is composed of a variety of different molecules (30,31).

Molecular transportation on i-BRB occurs through two routes, namely (1) a variety of paracellular routes during the transport process, opening and closing according to the needs of small molecular networks and solutes (2), the transcellular route includes vesicular transporters that exist in all endothelial cells and selectively

regulated by cell membrane transporters. i-BRB also includes different structural cells around endothelial cells, which also act as a barrier, such as pericytes, macroglial cells such as astrocytes, Müller cells, and microglial cells. In addition, the pericytes and endothelial cells are surrounded by basal cell membranes which contribute to i-BRB. In the condition of diabetes, both obstacles experience metabolic disorders because different molecules are formed in a hyperglycemic environment (30,31).

3.3 The role of tight junction, Müller cells, and microglial cells

Tight junctions are composed of complex protein aggregates, including zonula occludens-1(ZO-1), zonula occludens (ZO-2), and zonula occludens-3 (ZO-3). In experimental studies, the diabetes model shows tight junction protein disorganization, as shown by decreasing occludent content in retinal endothelial cells which causes reversible increases in permeability (26,31,32).

It is known that cells around the retinal capillaries, especially astrocytes, play an important role in the induction and maintenance of BRB function. The extent to which primary neuroglial (Müller cells) dysfunction plays a role in solving BRB is still being investigated. It is said that retinal bleeding is involved in macular edema. This hypothesis states that the enzymatic cascade initiated by carbonic anhydrase released by erythrocytes can increase levels of local bradykinin, which is a powerful vasodilator. This enzymatic cascade involves a decrease in local pH due to the release of $HCO3^-$. The next process that occurs is the activation of factor XII, increased levels of kallikrein, and finally the transformation of kininogen into bradykinin (26,33).

3.4 Blood Retinal Barrier (BRB) Damage.

BRB isolates the neuroretina element from the circulation to maintain the influence of the extracellular environment. i-BRB schematically consists of an intercellular barrier (tight junction that is between adjacent endothelial cells) and a transcellular barrier. Plasma can flow between endothelial cells through opening tight junctions or endothelial cells due to increased membrane permeability or vesicular transport. BRB dysfunction can be seen in the presence of microaneurysms. However, fluorescein leaks are also found in capillaries that appear "normal". This shows that BRB dysfunction occurred earlier than the morphological changes of capillaries (26,33).

BRB damage is a cause of macular edema which can occur in several ocular abnormalities and can be caused by structural changes or dissolved mediators. BRB damage is not caused by a single factor but is a complex process that involves many factors, receptors, and signalling pathways. Changes in the composition, distribution, or phosphorylation of proteins can result in leakage of blood vessels through tight junctions. BRB damage can also be caused by increased trans-endothelial vesicular transportation. Many of the same mediators simultaneously cause the tight junction to open and increase vesicular transportation. Pericytes and perivascular astrocytes that regulate the inner BRB (i-BRB) can also cause BRB damage, besides degenerative or structural changes in RPE cells. In addition, inflammation can also cause damage to BRB, so the use of anti-inflammatories, in this case, provides benefits (26,33).

4. The current issues microRNA, Post Translation Role

In addition to changes in metabolism, the condition of hyperglycaemia also causes epigenetic changes that are based on enzymatic processes, their manifestations resemble those due to changes in gene expression but without a picture of changes in DNA sequencing (34). Epigenetics is a challenging area, where the DM environment will support the occurrence of epigenetic modification, consisting of gene modification that plays a role in the pathogenesis of DR and enzymes that are responsible for the modification of epigenetic and altered miRNA in the retina of patients with DM. All of the above factors reinforce the notion of the role of post-translation modification in the progression of the DR. These various mechanisms work closely together and form a network that causes pathological changes in the retina including disorders of vascular integrity, increased vascular

permeability, and neovascular retinal formation (NV) (34).

Among several pathways of the DR pathogenesis mechanism, protein-encoding genes in VEGF and pigment epithelium-derived factor (PEDF) have an important role in vascular changes. Besides the large role in the infrastructure and maintenance of the DR process by protein-coding genes such as t-RNA and r-RNA, the no-protein coding RNA (ncRNA) is divided into microRNA (miRNA) and long non-coding RNA (lncRNA) based on their origin, structure, and biological function also have a complementary role in DR process (35).

The miRNA plays an important role in the proliferation, migration, and apoptosis of human cells, including retinal cells. It is not surprising that miRNA has been reported to play an important role in regulating NV associated with DR. miRNA is a short-chain (21-23 nucleotides) and is dense in endogenous sequencing of RNA that does not encode proteins, miRNA modulates gene expression through transcription and post-transcription pathways, degrades mRNA and inhibits the process of protein translation by binding to growth areas 3 'UTR of targeted genes. And found in all cell types in humans and is involved in almost all biological processes such as growth, differentiation, and apoptosis (18). miRNA also approved target genes by binding to 3' UTR mRNAs, post-transcription induction in gene correction (12,36). MicroRNAs (miRNAs) are also referred to as the 'micromanagers of gene expression,' miRNAs are evolutionarily well-conserved and, by binding to the target transcript in the 3'-UTR, can inhibit the translation of proteins and destabilize their target mRNAs. Predicted to regulate almost a third of the human genome, miRNAs are essential for cellular and organism development. The discovery of miRNAs, encoded in what was previously considered 'junk DNA', as master regulators of gene expression have revealed that the term 'junk DNA' is a misnomer (37).

Primarily a miRNA consists of the 5'-region of a miRNA, from positions 2-7, called the 'seed' region, which undergo two subsequent cleavages by the RNase III enzymes, Drosha, within the nucleus, and Dicer, after translocation into the cytoplasm, resulting in the formation of double-stranded, mature miRNAs. This sequence contains mature miRNA and its passenger strands. The guide strand then joins one of the Argonaute (Ago) proteins to form an RNA-induced silencing complex (RISC); the passenger strand is then discarded and degraded. Mature RISCs can bind UTRs, generally 3'-UTR. The so-called seed region extends between nucleotides 2 and 7, from the 5' end region of the adult miRNA, binding to the complementary sequence in the target 3'-UTR mRNA in a Watson–Crick complementarity. These complementary sequences, positioned between the seed region and the mRNA binding site at 3'-UTR, are needed for target recognition. However, several studies have shown that targeting miRNA is not always limited to 3'-UTR, with respect to 5'-UTR mRNA. There are natural examples of miRNA that regulate mRNA expression in a seed-dependent manner. Finally, the miRNA-mRNA bond leads to inhibition of translational or degradation of the target mRNA and leads to the negative regulation of protein synthesis or mRNA degradation. It is clear that biological effects on cells occur through the repression of specific proteins involved in certain biological pathways. A single miRNA can modulate the expression of multiple mRNAs; conversely, more than 60 % of mRNA has estimated binding sites for multiple miRNAs, thus allowing simultaneous interaction with multiple miRNAs. Therefore, it is hoped that the function and biogenesis of miRNA can be regulated, because their changes are related to various human diseases, including chronic conditions (38).

The human genome contains genes encoding 1 000 miRNAs. miRNAs bind to their targets based on nucleotide sequence regions of mRNA, as well as genomic DNA and have a "one-to-many and many-to-one" relationship with their targets and can potentially influence the expression of nearly all protein-coding genes (10). We still have little knowledge of which miRNA is involved in the onset and progression of diabetic retinopathy. A small number of studies have identified the expression and functions of specific miRNA in diabetic retinopathy (39,40). Computational analyses have been done to find regulatory networks of miRNA, but the majority of miRNA targets have not been experimentally tested. Moreover, little has been shown about the regulatory networks of miRNA in diabetic retinopathy (12).

5. NEW PERSPECTIVE OF DME MANAGEMENT BASED ON NON-CODING RNA PATHWAY (MICRORNA)

The level of complementarity with the mRNA target determines which silencing mechanism will be employed; cleavage of target messenger RNA (mRNA) with subsequent degradation or translation inhibition. Although the biological function of identified miRNAs may be unknown, examination of the expression profiles of these molecules provides information on their regulation and function. Such observations have indicated that miRNA expression profiles are altered in specific tumors, implying that miRNA may be involved in the development of cancer and other diseases (41).

miRNA pharmacogenomics can be defined as the study of miRNAs and polymorphisms affecting miRNA function to predict drug behavior and improve drug efficacy. Advancements in the miRNA field indicate the clear involvement of miRNAs and genetic variations within the miRNA pathway in the progression and prognosis of diseases such as cancer, neurological disorders, muscular hypertrophy, gastric mucosal atrophy, cardiovascular disease, and Type II diabetes. Generally, miRNAs regulate the gene expression of a target gene by binding to its 3'-UTR. MiRNAs can potentially regulate the expression of multiple genes and pathways; for example, it has recently been shown that the miR-15a/16–1 cluster can directly or indirectly regulate the expression of approximately 14 % of known human genes. More and more evidence suggests that a gain or loss of miRNA function is associated with disease progression and prognosis. Several studies have now established that miRNAs are differentially expressed in human cancers as compared with normal tissue (37).

Amongst the pathways participating in the pathogenesis and progression of DR, a large number of related protein-encoding genes have been identified and shown to play crucial roles in vascular changes. These factors include vascular endothelial growth factor (VEGF), pigment epithelium-derived factor (PEDF), angiopoietin, and bone morphogenetic protein (BMP). In recent decades, miRNAs, have been broadly studied in the different pathways involved in the pathogenesis of DR (42). There may be other potential mechanisms through which miRNA directly targets signaling molecules that are related to pathological pathways of diabetic retinopathy, such as mitogen-activated protein kinase (MAPK) family, matrix metallopeptidases (MMPs), integrin, and others (targetscan.org).

The ultimate goal of studying miRNA in association with diabetic retinopathy would be to develop novel therapeutic strategies using potent miRNA for the treatment of the diabetic retina. *In vivo* research must be performed to prove the protective effects, safety, and efficiency of miRNA-based therapy. A small number of studies have been done for *in vivo* delivery of miRNA in animal models, in which an intravitreal injection was done to deliver miRNA into the eyes of diabetic mice and rats. Those studies have shown inhibitory effects of miRNA on retinal neovascularization (miR-31, -150, and -184, miR-126, anti-miR-155, miR-184, miR-218), fibronectin (miR-146a), VEGF (miR-200b), and SIRT1(miR-23b-3p and miR-195) (12,43,44).

Table 5.1 miRNAs involved in the Vascular Smooth Muscle Cells (VSMC) (12,43,44,54)

	Targets	Main effect
miR 15a	IL-1; TNF; NF-kBp65	Anti-inflammatory
miR 23b/195	SIRT1; SEMA6A; SPROUTY2	Pro-angiogenic
miR 31	LATS2; CRE6	Anti-angiogenic
miR 126	PI3KR2; SPRED1;	Pro-angiogenic
	VCAM1; SDF1	Anti-inflammatory
miR 133a	MSN; SP1	Anti-angiogenic
miR 146a	HuR; NF-kB	Anti-angiogenic
miR 200	Ets-1; IL-8; CXCL1	Anti-angiogenic
miR 221/222	STA5a; c-KIT; eNOS	Anti-angiogenic

High glucose conditions and hyperglycemia can initiate leukostasis, a key to inflammatory change, and may represent the first step in diabetic retinopathy. Retinal leukostasis is regarded as a histological indication of retinal inflammation. We generated conditional knockout mice in which miR-15a/16 was eliminated in vascular endothelial cells and found that retinal leukostasis could be attenuated by miR-15a/16. Specifically, we showed that miR-15a/16 played a role in reducing the influx of CD45+ leukocytes in the retina, with our outcome excluding the possibility that the regulatory effects were induced by potential changes due to the number of circulating pool of leukocytes (45). Further investigations will be required to gain a better understanding of the regulatory functions of miR-15a/16 in diabetic retinopathy.

miR-15a/16 played a role in reducing pro-inflammatory signalling of IL-1β, TNFα, and the phosphorylation of NF-κBp65 (ser536) in cultured REC. The role of miR-15a on inflammatory pathways of diabetic retinopathy, whereby miR-15a played a role in suppressing pro-inflammatory pathway through inhibition of sphingomyelinase in sphingolipid metabolism. Other than miR-15a and -16, only a small number of miRNAs have been tested for their regulatory roles in pro-inflammatory pathways of diabetic retinopathy (12).

Table 5.2 miRNAs involved in Proliferative; de-defferentiated states (54)

	Targets	Main effect
miR 21	PTEN, Bcl-2	Proliferative, de-differentiate
miR 24	TRB3	Proliferative, de-differentiate
miR 26	SMAD1/4	Proliferative, de-differentiate
miR 126	PI3, KR1, SPRED1, VCAM1, SDF1	Proliferative, de-differentiate

In addition on miR15a/16 on retinal leukostasis, miR-126 was shown to regulate vascular cell adhesion molecule 1 (VCAM-1) expression and inhibit leukocyte adhesion in the retinas of diabetic rats. Additionally, an inhibitory role of miR-146 in thrombin-induced increased leukocyte adhesion to human REC and the relation of miR-335 to leukocyte activation were also found in the retina of streptozotocin (STZ)-diabetic rats. MicroRNA-126 (miR-126) is an 85-bp mature circulating microRNA that has been shown to regulate endothelial cell responses to vascular endothelial growth factor (VEGF). MicroRNA-126 (miR-126) is thought to be involved in the process of neovascular disease by promoting VEGF and reducing the inflammatory response by inhibiting vascular cell adhesion molecules 1 (VCAM1) (46,47).

Micro-RNA-126 (miR-126) is located in the seventh gene such as protein 7 (EGFL7) epidermal growth factors. Proteins such as epidermal growth factor 7 (EGFL7) are located on chromosome 9q34.3. McAuley et al. (2015) provided the effect of EGFL7 SNP intronic (rs4636297) in the cohort of the Caucasian race, and they found a significant relationship with the development of DR. This variant is expected to allow maturing miR-126 to circulate but does not require VEGF circulation, this is considered to increase VEGF production and increase the risk of DR (38).

MicroRNA-146a (miR-146a) is involved in the innate immune system. The encoding for the miR-146a gene is located on chromosome 5q33.3. Mature miRNA is inserted into the RNA-induced junction complex (RISC) and results in translational inhibition or destabilization of the target mRNA. Micro-RNA-146a (miR-146a) is a mediator of inflammation and miR-146a expression is regulated by Interleukin (IL)-1 and tumor necrosis factor-alpha (TNF-α). The miR-146a target is involved in receptor pathways such as Toll (TLR4) that cause cytokine responses. MicroRNA-146a (miR-146a) operates in a feedback system to smoothly regulate the immune response. Kaidonis et al. in 2016 analyzed the polymorphism of rs2910164 in miR-146a in a cohort of Caucasian patients with T2 DME. They found an association between rs2910164 and DME (19,48,49). MiR-146a is one of many candidates, in addition to miR-15b-, -16, -18b,

-29, -195, and -200b, -221, that have been studied for a regulatory role in diabetic retinopathy and/ or hyperglycemia (12).

TGF-β signalling pathway is also under the control of miRs. TGF-β superfamily of growth factors triggers Vascular Smooth Muscle Cells (VSMC) differentiation by post-transcriptionally increasing the expression of a subset of miRs including miR-21. miR-24 is involved in both TGF-β and PDGF-BB signaling pathways, which respectively represent the distinguishing trigger of VSMC differentiation and proliferation. Inhibition of miR-26a promotes VSMC apoptosis and phenotypic switch to a contractile status while inhibiting proliferation and migration (49).

Macconi et al. added miR-324-3p into the interesting mix of miRNA regulators in renal fibrosis. They found miR-324-3p was significantly upregulated in glomeruli from the kidneys of Munich Wistar Fromter (MWF), acknowledgment rats with spontaneous progressive nephropathy (10). This study strongly suggests that dysregulation of the miR- 324-3p/ Prep complex contributes to the progression of spontaneous nephropathy in MWF rats. However, its universal validity remains to be clarified, because miR-324-3p has never been identified in any lists of upregulated miRNAs detected in kidney tissues from either diabetic nephropathy or IgA nephropathy in which the profibrotic effects of ACE inhibition have been confirmed (10).

The other issues are polymorphism, rs3025040 T allele significantly decreased the luciferase activities in four cell lines, which indicated a potential disruption of the miRNA-mRNA interaction that would result in lower VEGF expression levels. Since both +936 C > T and +1451 C > T were located in the 3'UTR of the VEGFA gene, we hypothesized that these genetic variants may interrupt miRNA-mRNA interactions and affect VEGF expression. We identified miR-591 and miR-199 families as containing seed sequences that respectively corresponded to the complementary sequences surrounding +936 C > T and +1451 C > T. Our data suggested that the +936C/T variants significantly increased the risk of poorer stroke outcome by affecting the bindings of miR-199a and miR-199b to VEGF mRNA at the rs30250340 polymorphic site (50). It was also demonstrated in plants, (Arabidopsis and related Brassicaceae), that mutations in the miRNA itself resulted in the loss of miR-319a function, which was further compensated by other members of the miR-391 family (37).

6. miRNA 126 AND ITS ROLE IN DME AND THE RELATED MECHANISM

Diabetic macular edema (DME) is thought to occur because of disruption of the blood-retina barrier (BRB), which leads to swelling or thickening in the macular area as a result of the accumulation of sub- and intraretinal fluid in the inner- and outer plexiform layers (20,21). Inflammation likely plays a key role in some subtypes of DME pathogenesis, and the observation that the phenotype closely resembles that of cystoid macular edema (CME) seen in uveitis and Irvine–Gass syndrome (51,52) supports this.

MicroRNA-126 (miR-126) is an 85-bp mature circulating microRNA that has been shown to regulate the response of endothelial cells to vascular endothelial growth factor (VEGF) (see Vascular endothelial growth factor (VEGF)) (Fish et al., 2008). MicroRNA-126 (miR-126) is thought to be involved in neovascular disease processes by promoting VEGF as well as reducing the inflammatory response by inhibiting vascular cell adhesion molecule 1 (VCAM1) (47,53). MicroRNA-126 (miR-126) is located within the seventh intron of the epidermal growth factor-like protein 7 (EGFL7) gene. Epidermal growth factor-like protein 7 (EGFL7) is located on chromosome 9q34.3. McAuley et al. (2015) investigated the effect of the intronic EGFL7 SNP (rs4636297) in their Caucasian cohort, they found that there was a significant association with the development of DR. This variant is thought to allow mature miR-126 to circulate but does not regulate circulating VEGF, this is thought to increase VEGF production and hence increase the risk of DR (19).

This miR plays a critical role in modulating vascular development and homeostasis, targeting specific mRNAs including the Sprouty-related protein 1 (SPRED-1), CXCL12, SDF-1, and phosphoinositol-3 kinase regulatory subunit 2 (PIK3R2). Confirming its key role in maintaining vascular integrity, amidst the other targets of

miR-126 there is a key mediator of leukocyte adhesion and inflammation: vascular cell adhesion molecule 1 (VCAM- 1). miR-126 has also been related to the endothelial dysfunction associated with the development of diabetes and its complications (54).

The levels of miR126 and VEGF-C expression gradually decreased, while the level of spred-1 expression significantly increased during the induction process. MiR-126-binding sites in spred-1 mRNA were predicted by computational algorithms and verified by the luciferase reporter assay. The use of miR-126 mimics revealed reduced spred-1 levels and increased VEGF levels. However, the mechanism by which miR-126 mediates the angiogenesis process during the development of RNV remains under investigation (55).

MicroRNA-126(miR-126) targets involved in inflammation need to be identified. Several studies aimed to investigate whether high-mobility group box 1(HMGB1), an inflammation-related gene, is the target of miR-126 in diabetic vascular endothelium. MiR-126, an endothelial cell-restricted miRNA that mediates vascular development and angiogenesis, has been considered as an important therapeutic implication for a variety of diseases involving aberrant angiogenesis and vascular leakage. Although several miR-126 targets associated with the inflammation process have been identified, need further exploration for underlying targets. Several studies have found that HMGB1 was a target of miR-126 in diabetic mice used as a classical diabetic atherosclerosis model. Intriguingly, miR-126 could suppress inflammation in high glucose incubated endothelial cells by modulating the expression of HMGB1 (56).

The high-mobility group box 1 (HMGB1) protein, a nuclear non-histone DNA-binding protein remarkably elevated in many inflammatory diseases, involves in the pathogenesis of Cardiovascular Disease (CVD) in DM. PCR was carried out using SYBR green (Takara Bio., Inc., Otsu, Japan) on a real-time PCR system using the specific primers for HMGB1. The mRNA level was normalized by GAPDH. The amplification results were calculated as $2(-\Delta\Delta Ct)$, according to the previous description mRNA-HMGB1 (56). A possible explanation of the ability of miR-126 to specifically target Ang-1/Tie-2 signaling comes from the analysis of its target genes. Many genes have been postulated to be regulated by miR-126, including VCAM, EGFL7, IRS-2, HOXA9, PAK1, and c-Myb. However, conclusive results have only been obtained for SPRED1, while experiments with p85β produced contrasting evidence (53).

Endothelial cells (EC) of mature vessels express high levels of miR-126, which primarily targets phosphoinositide-3-kinase regulatory subunit 2 (p85β). Down-regulation of miR-126 and over-expression of p85β in endothelial cells inhibit the biological functions of Ang-1 (Angiopoietin-1). Additionally, the knockdown of miR-126 in zebrafish resulted in vascular remodelling and maturation defects, reminiscent of the Ang-1 loss-of-function phenotype. Our findings suggest that miR-126-mediated phosphoinositide-3-kinase regulation, not only fine-tunes VEGF-signalling but strongly enhances the activities of Ang-1 on vessel stabilization and maturation (53).

Ang-1 is necessary for subsequent vascular remodelling into mature blood vessels Ang-1 stabilizes existing vessels and decreases vascular leakage. Recent studies highlighted the additional beneficial roles of Ang-1 in cancer models. In contrast, the overexpression of angiopoietin-2 (Ang-2) was associated with an advanced disease state and poor prognosis in different solid malignancies, thus suggesting differential roles of Ang-1 and Ang-2 in tumor biology. Ang1 is a potent angiogenic growth factor signalling through Tie2 (receptor tyrosine kinases =RTKs), whereas Ang2 was initially identified as a vascular disruptive agent with antagonistic activity through the same receptor (57). In the presence of TNF-alpha, EC released a higher level of miR-155/MP but tremendously decreased the level of miR-126 and miR-21/MP. Alexy et al. examined the formation of miR-155 encapsulated micro-vesicles (MP) by endothelial cells (EC) following TNF-alpha treatment (58).

miR-126 has been extensively studied in plasma and circulating cells because its expression is very high in EC, endothelial progenitor cells (EPCs), and platelets. Most recently, miR-126 has been identified as an efficient marker in the detection and purification

of EC (54). A significant increase in circulating miR-126 has been detected in patients with acute myocardial infarction and angina whereas miR-126 downregulation has been reported in plasma from patients with diabetes, heart failure, or cancer (54).

Investigation of the content of serum microRNA-126 (miR-126) and its role in screening retinal endothelial injury (NPDR) and early diagnosis of proliferative diabetic retinopathy revealed serum content of miR-126 declined as the damage degree increased in the retina. Receiver operating characteristic curve (ROC) analyses indicated that serum miR-126 had a significant diagnostic value for PDR. When the diagnostic threshold was greater than or equal to 8.43, there was an increase in the possibility of NPDR. When the content of miR-126 was less than or equal to 5.02, the possibility of the occurrence of PDR increased (59).

Significant decrease of serum miRNA-126 expression between impaired glucose tolerance (IGT) and diabetic patients when both compared to controls as well as between diabetic patients compared to IGT patients. Circulating concentrations of serum miRNA-126 were negatively associated with both fasting glucose, HbA1c, and 2hpp blood glucose. In addition, low miR-126 expression was significantly associated with poor treatment response (60).

A large cohort study of type 1 diabetic subjects also found that miR-126 levels are associated with vascular complications of diabetes, particularly with proliferative retinopathy. The inverse associations between miR-126 and vascular complications remained only marginally significant after adjustment for A1C and diabetes duration, suggesting a major role of hyperglycemia in mediating the relationship between miR- 126 and both micro- and macrovascular complications of diabetes, it reduces miR-126 relevance as a potential new clinical biomarker for early diagnosis of complications (61).

MiR-126 is highly abundant in endothelial cells and plays a pivotal role in maintaining endothelial homeostasis, vascular integrity, and regulating angiogenesis. It helps vascular endothelial growth factor (VEGF) signalling by suppressing two negative regulators of the (VEGF) pathway, Sprouty-related protein (SPRED1) and phosphoinositol-3 kinase regulatory subunit 2 (PIK3R2/p85-b). The shedding of miR-126 from endothelial cells could regulate VEGF responsiveness and confer vascular protection in a paracrine manner (62).

CONCLUSION

MicroRNA-126 (miR-126) is thought to be involved in neovascular disease processes by promoting VEGF as well as reducing the inflammatory response by inhibiting vascular cell adhesion molecule 1 (VCAM1). The levels of miR126 and VEGF-C expression gradually decreased, while the level of spred-1 expression significantly increased during the induction process. The use of miR-126 mimics revealed reduced spred-1 levels and increased VEGF levels.

MicroRNA-126(miR-126) targets involved in inflammation need to be identified. Some studies aimed to investigate whether high-mobility group box 1(HMGB1), an inflammation-related gene, is the target of miR-126 in diabetic vascular endothelium. miR-126-mediated phosphoinositide-3-kinase regulation, not only fine-tunes VEGF-signalling but strongly enhances the activities of Ang-1 (Angiopoietin -1) on vessel stabilization and maturation. Ang-1 is necessary for subsequent vascular remodelling into mature blood vessels.

At the end of conclusion, miRNA 126 is a good choice in preventing the development of diabetic macular edema and proliferative DR, by both mechanism (angiogenesis and inflammation role) and links between angiogenic miRNA expression profiles, Ang (Angiopoietin) and immune-cell responses by TEMs (TIE2- expressing monocytes) have clinical implications as novel diagnostic tools with regards also to prognostic significance.

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Conflicts of interest

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