Review

Relationship between Biochemical Pathways and Non-Coding RNAs Involved in the Progression of Diabetic Retinopathy

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Relationship between Biochemical Pathways and Non-Coding RNAs Involved in the Progression of Diabetic Retinopathy

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Abstract: Diabetic retinopathy (DR) is a progressive blinding disease that affects vision and quality of life of patients and severely impacts society. This complication, caused by abnormal glucose metabolism, leads to structural, functional, molecular, and biochemical abnormalities in the retina. Oxidative stress (OS) and inflammation also play pivotal roles in the pathogenic process of DR, leading to mitochondrial damage and decrease in the mitochondrial function. DR causes retinal degeneration in glial and neural cells, while the disappearance of pericytes in retinal blood vessels leads to modifications in vascular regulation and stability. Clinical changes include dilatation and blood flow alterations in response to the reduce in retinal perfusion in retinal blood vessels, leading to vascular leakage, neovascularization and neurodegeneration. Thus, DR is a highly complex disease with various biological factors that contribute to its pathogenesis. The interplay between biochemical pathways and non-coding RNAs (ncRNAs) is essential for understanding the development and progression of DR. Abnormal expression of ncRNAs has been confirmed to promote the development of DR, suggesting that ncRNAs such as miRNAs, lnc-RNAs and circ-RNAs have potential as diagnostic biomarkers and theranostic targets in DR. This review provides an overview of interactions between abnormal biochemical pathways and dysregulated expression of ncRNAs under the influence of hyperglycemic environment in DR.

Keywords: diabetic retinopathy; biochemical pathways; oxidative stress; ncRNAs; biomarkers

Introdution

In recent years, a systematic increase in the incidence of diabetes mellitus (DM) has been observed, and this disease is considered a global epidemic. According to estimates, in 2021, around 537 million people (aged 18–99) in the world suffered from DM. Almost half (49.7%) of all diabetic patients are still undiagnosed. It is projected that by 2045 number of patients with DM will have increased to approx. 783 million [1].

DM is accompanied by many complications, which are the main cause of chronic diseases, including eye diseases. DM can lead to many serious ocular complications, including diabetic retinopathy (DR), neovascular glaucoma (NVG), cataracts and ocular surface changes. Due to poor glycemic control and increasing insulin resistance, diabetic retinopathy, being one of the main microvascular complications, is becoming the leading cause of blindness in the population of professionals in urban areas [2]. The longer the duration of diabetes, the more often DR occurs. Epidemiological studies estimate that 20 years after the diagnosis of the disease, almost all patients with type 1 DM and in approximately 80% of patients with type 2 DM have demonstrated changes related to diabetic retinopathy [3]. Patients diagnosed with diabetes at a young age are at greater risk of retinopathy and its progression. Patients with type 2 diabetes develop retinopathy early, i.e. within 3 years following its diagnosis, 12% of people develop changes in the eye fundus. Due to the overall rapid increase in the incidence of diabetes, the number of adults worldwide with DR, vision-

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threatening DR, and diabetic macular edema (DME) is projected to increase to approximately 161 million, 45 million, and 29 million, respectively by 2045 [4].

Diabetic retinopathy (DR) is a complex microvascular disease characterized by various clinical manifestations and biochemical pathways that contribute to its pathophysiology. The exact mechanism responsible for the development and progression of diabetic retinopathy is not fully understood, but extensive research has shed light on several key involved molecular processes. Moreover, DR has long been recognized as a microvascular complication of diabetes. However, accumulating evidence points to neurodegeneration as an early event in its pathogenesis [5,6]. DR is a common and debilitating microvascular complication of both type T1DM and type T2DM. In fact, retinal dysfunction can be detected in diabetic subjects without evidence of microvascular abnormalities, and the American Diabetes Association (ADA) defines DR as a highly specific neurovascular complication. Thus, in view of current knowledge on microangiopathic changes in the eye fundus, diabetic retinopathy is perceived as a neurodegenerative disease [7,8]. Abnormalities in the structure and function of the retinal blood vessels in diabetic patients are manifested by deterioration of visual acuity, reduced contrast sensitivity, delayed dark adaptation and poorer color vision [9].

Factors That Increase Development of Diabetic Retinopathy

The development and progression of changes in diabetic retinopathy are associated with many factors. In the first place, the duration of the disease and the age at diagnosis of diabetes should be mentioned, as they are considered the main factors in the development of diabetic retinopathy. Furthermore, most diabetic patients undergo an eye check-up only when necessary. There is a very complex relationship between a person's socioeconomic status and the disease [10]. Fluctuations in blood glucose levels, which have a negative impact on the state of retinal microcirculation and may lead to rapid disease progression are the second risk factor [11]. In addition, a high level of glycosylated hemoglobin (HbA1c) may indicate progression of changes in the eye fundus [12]. Other factors that may contribute to development of retinopathy are: higher systolic or diastolic blood pressure, hypertension [13], obesity [14], hyperlipidemia [15,16], anemia [17], pregnancy [18], puberty [19], cataract surgery [20], nephropathy [21]. The waist-to-hip ratio (WHR), an indicator of central obesity, has been found to be associated with insulin resistance and serves as an independent risk factor for retinopathy in diabetic patients. Furthermore, a combination of WHR, Hb A1c level, and hypertension have been identified as risk factors for retinopathy [22]. DR and nephropathy have been shown to coexist in both type 1 and type 2 diabetes. DR may be the most common microcirculatory problem in diabetes, even more common than nephropathy [13].

Elevated blood pressure (BP) is also a recognised risk factor for DR progression in pregnancy (i.e. systolic BP >115 mmHg in pregnant women in comparison to BP <105 mmHg in non-pregnant women) [23].

In addition, investigations have revealed a significant association with sleep apnea [24], post-translational amendments of histones within chromatins [25], DNA methylation [26] and non-coding RNAs, such as miRNAs [27–29] and lncRNAs [30,31].They all affect many biochemical pathways.

Diabetic Retinopathy - Classification

Diabetic retinopathy is a microangiopathy affecting particularly the small vessels of the retina. In the course of diabetic retinopathy, symptoms of obstruction and leakage of capillaries predominate. Structural and functional changes occurring in individual layers of the retina in diabetic patients appear before clinical manifestations of DR. Reduced retinal blood flow is observed in early stages of the disease. Inflammatory changes, leukostasis, vascular occlusion and neurodegenerative changes lead to hypoxia and further disease progression. As a result of remodeling of vascular walls and changes in blood rheology, perfusion is reduced in retinal ischemia [32].

DR is usually asymptomatic at the onset. Early clinical features of DR are visible during an ophthalmoscopic examination at the fundus. Diabetic retinopathy can be divided into non-

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proliferative (simple) retinopathy (NPDR), affecting the retina and proliferative retinopathy (PDR), characterized with a loss of vision.

NPDR manifests by small haemorrhages in the middle layers of the retina, microaneurysms resulting from protrusion of a weakened retinal capillary wall, hard exudates formed by lipid material accumulating in the outer plexiform layer, areas of accumulation and stagnation of axoplasms of nerve fibers, lack of blood flow and intraretinal microvascular abnormalities (IRMA). Retinal edema may result from microcirculatory exudate and is an indicator of impaired blood-retinal barrier [33].

Proliferative diabetic retinopathy is associated with proliferation of pathological vessels - neovascularization within the retina and/or optic disc. PDR may lead to preretinal hemorrhage and/or vitreous hemorrhage. New vessels get to the surface of the retina and into the vitreous body through cavities in its inner layers. The vitreous body shrinks, which additionally causes hemorrhages, contributing hereby to developing vascular-fibrous proliferation. In consequence, a part of the neurosensory retina gets detached from its pigment epithelium. Without medical intervention, vision loss will occur because neurodegeneration will contribute to neuroapoptosis, which in turn, will lead to death of retinal ganglion cells, glial modifications, and deviations in the retinal pigment layer. Furthermore, neovascularization may develop in the anterior segment of the eyeball, on the iris, as a result of severe changes in the eye fundus and intensive production of vascular endothelial growth factor (VEGF) [6].

Involvement of Biochemical Changes in Progression of DR

- Hyperglycemia and Hypoxia

Many metabolic pathways are altered by high glucose (HG) levels. Some of major pathways implicated in the development of retinopathy include increased activity of the diacylglycerol (DAG) pathway and protein kinase C (PKC), accumulation of advanced glycation end products (AGEs), activation of the hexosamine pathway, overproduction of growth factors: vascular endothelial growth factor –(VEGF) and insulin-like growth factor (IGF-1), activation of the renin-angiotensin-aldosterone system (RAAS), accumulation of inflammatory mediators and subclinical inflammation with leukostasis, activation of the polyol pathway and oxidative stress [34].

At the onset of pathomechanism, high glucose level contributes to microvascular degeneration and disruption of the blood-retinal barrier (BRB). Endothelial cells of the retinal vessels constitute a tight barrier for blood components. Hyperglycemia causes many alterations in endothelial cells and their supporting cells - pericytes, which results in their dysfunction and death. Pericyte and endothelial cell apoptosis, capillary occlusion, and elevated vascular permeability also occur during the early phase of NPDR. Any changes causing e.g. a formation of microaneurysms reduces the efficiency of supply of oxygen and nutrients to retinal cells. As a result of oxygen deficiency, deepening hypoxia stimulates the production of growth factors responsible for formation of new blood vessels, i.e. angiogenesis [35,36].

- Disorders of the Polyol Pathway

Hyperglycemia also causes increased conversion of glucose to sorbitol and fructose via the polyol pathway and overexpression of enzymes that are part of this pathway: aldose reductase (AR) and sorbitol dehydrogenase (SDH). These processes occur in tissues where glucose transport is independent of insulin (retina, eye lens, kidneys, peripheral nerves). The polyol pathway is activated in endothelial cells, pericytes and Müller cells to produce macular edema and subsequent retinal ischemia. The concentration of sorbitol, fructose, galactitol increases, which poorly penetrate cell membranes, accumulate, which leads to swelling and tissue damage. Increased oxidation of $NAPDH^-$ (nicotinamide adenine dinucleotide phosphate) to $NADP^+$ and reduction of NAD^+ to NADH leads to tissue hypoxia [37]. Sorbitol, impermeable to cellular membranes, gets accumulates within the cell. This phenomenon is followed by slow metabolism of sorbitol to fructose. Fructose produced by the polyol pathway can be phosphorylated to fructose-3-phosphate, which in turn, can be degraded to 3-deoxyglucosone. These two are strong glycating agents and can contribute to production of AGEs

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[38–40]. Studies have shown that increased AR activity is localized in, among others, pericytes, retinal endothelial cells, ganglion cells, Müller cells, retinal pigment epithelial cells and neurons. AR hyperactivity is involved in destruction of retinal cells [41]. Furthermore, the aldose reductase gene is characterized with the largest number of polymorphisms associated with DR [42].

- The diacyloglycerol and Kinase Pathway Protein C

Activation of glucose metabolism via the polyol pathway also increases the concentration of diacyloglycerol (DAG). Under hyperglycemic conditions, DAG may be formed de novo from intermediate products of glycolytic metabolism. A high concentration of diacylglycerol activates retinal cellular protein-kinase C (PKC). On the one hand, DAG can directly activate PKC in the retina, mainly β- and δ-type isozymes, while the activation of PKC-α and -ε is also found in the retina of diabetic rats [43]. On the other hand, AGE-mediated signaling pathways as well as metabolic products of the polyol pathway are also associated with PKC activation. Increased PKC activity contributes to overexpression of matrix proteins and vasoactive mediators. It causes adverse retinal vascular changes, involving structural ones, such as pericyte apoptosis, basement membrane thickening as well as functional ones, such as increased retinal vascular permeability and retinal blood flow [44,45]. Pericytes degeneration begins with the progressive stimulation of PKC-δ signaling, induced by hyperglycemia. Signal enhancement stimulates the expression of PKC-δ and p38 mitogen-activated protein kinase. It induces dephosphorylation of platelet-derived growth factor receptors (PDGFRs) and reduces its downstream signaling, which in turn results in self-mediated death of pericytes [46]. In addition, the activation of PKC may cause the overexpression of endothelin-1 (ET-1), being a potent vasoconstrictor, which seems to be involved in the pathogenesis of DR and can also explain the decreased retinal blood flow [47].

- Growth Factors, Cytokines and Angiogenic Factors

Diabetes leads to reduced cellular proliferation [36] and endothelial cells dysfunction, hereby causing defective angiogenesis [35]. Increased activity of growth factors is an important mechanism in development of DR.

Several pro-angiogenic cytokines, including vascular endothelial growth factor (VEGF), insulin-like growth factor I (IGF-1), hepatocyte growth factor (HGF), basic fibroblast growth factor (b-FGF), platelet-derived growth factor (PDGF), pro-inflammatory cytokines such as IL-1β [48], Il-6 [49], IL-8, tumor necrosis factor-α (TNFα) [50,51] and angiopoietins [52] are believed to be involved in the pathogenesis of PDR. Endothelial failure due to pericyte loss induces hypoperfusion that conducts to abnormalities in the structure and function of retinal blood vessels. These changes lead to sight-threatening points, which are main complications of diabetic retinopathy. Impairment of endothelial cells, the occurrence of cotton wool patches, microaneurysms, and dot and blot hemorrhages is associated with damage to pericytes. The pericyte loss and endothelial failure conducts to occlusion of capillaries and local ischemia, which induces hypoxia-inducible factor 1 (HIF-1). HIF-1 as triggering factor additional enhances the expression of VEGF, which along with the other angiogenic indicators, i.e., Ang-1 and Ang-2, increases vascular permeability [53]. In the terminating phase of the DR pathomechanism, hyperglycemia induces neovascularization and neurodegeneration processes [6]. The process of neurodegeneration is a consequence of high glucose level-induced suppression of vital neurotrophic and neuroprotective factors, including nerve growth factor (NGF) [54], pigment epithelium-derived factor (PEDF) [55], interphotoreceptor retinoid-binding protein (IRBP) [56], somatostatin (SST) and glucagon-like peptide 1 (GLP-1) [57], whereas neovascularization relates to the stimulation of pro-angiogenic biomarkers, such as VEGF, HGF, PDGF, Ang-1 and Ang-2.

- Oxidative Stress

The retina exhibits enhance concentrations of free radicals [58,59]. Their overproduction results from the oxidation of polyunsaturated fatty acids (PUFA) in the retina and phototransduction, as well as phagocytosis of the outer segments of photoreceptors by retinal pigment epithelium (RPE) cells [60]. Exposure to sunlight and ultraviolet rays that shine directly on the lens and retina of eye can cause retinal degeneration. In photo-oxidation processes, an ocular chromophore assimilates

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light and generates ROS such as singlet oxygen, superoxide, and hydroxyl radical, which induce lipid peroxidation, initiating damage to the integrity of the cellular membranes, thereby damaging ocular tissue.

Oxygen-consuming mitochondria in the photoreceptor inner segments also mean an important function in retinal free radicals overproduction. The retinal hypoxic and hyperglycemic conditions contribute to overexpression of NADPH oxidase (Nox) which leads to overproduction of free radicals, superoxides and hydrogen peroxide by taking electrons from NADPH and transporting them to molecular oxygen. The Nox system is involved in many processes leading to the overproduction of ROS. For example, the high blood glucose level increases formation of AGEs and binding of glycation end products to their receptor (RAGE), which results in increased production of intracellular free radicals via NADPH oxidase [61]. Moreover, activation of protein kinase C, via diacyloglycerol formation, results in subsequent ROS production also via Nox [62].

The research showed that Nox system is an important source of oxidative stress in the vascular system. It has been shown that increased Nox 2 activity is associated with ROS overproduction and overexpression of intercellular adhesion molecule-1 (ICAM-1) and VEGF [63].

In contrast, Brownlee and co-authors observed that mitochondrial-derived ROS causes strand breaks in DNA, which in turn activates poly-(ADP-ribose)-polymerase (PARP). High activation of PARP enzyme inhibits glyceraldehyde phosphate dehydrogenase (GAPDH) activity which intensifies the accumulation of glycolytic metabolites. The metabolites then activate the AGEs, PKC, polyol, and hexosamine pathways. The authors have proposed that many biochemical and pathological retinal abnormalities induced by diabetes are associated with oxidative stress [64].

It is suggested that the metabolic memory phenomenon and the mitochondrial DNA (mtDNA) damage by ROS might be potentially responsible for a prolonged progressive course of DR. In consequence, mtDNA damage generates subnormal complex I and III with reduced membrane potential. This causes the positive feedback loop where hyperglycemia induces superoxide, which damages mtDNA. This impedes the electron transport chain and results in superoxide overexpression [65].

Research has shown that damage to mitochondrial DNA (mtDNA) is not uniform, but specific regions aremore vulnerable than others. In particular, the displacement loop region (D-loop) of mtDNA, which is responsible for replication and transcription, is exposed to more extensive damage in the course of diabetic retinopathy [66,67]. Mitochondrial DNA methyltransferase (DNMT) is also highly expressed in retinal cells under high glucose condition, which can highly methylate the D-Loop region of mtDNA and cause mitochondrial damage in retinal cells [68]. Moreover, the mtDNA replication/repair system is compromised in DR, leading to decreased gene transcripts and mitochondrial accumulation of enzymes such as DNA polymerase gamma 1 (POLG1), DNA polymerase gamma 2 (POLG2), and mtDNA helicase. This impairment further hampers the replication/repair process and contributes to dysfunctional mitochondria. Copy numbers of retinal mitochondria are also decreased in diabetic retinopathy [65,69,70]. The D-loop integrity and the mtDNA replication play important roles in the pathogenesis of diabetic retinopathy, and the high glucose level preferentially damages the D-loop region and the replication machinery in the retinal endothelial cells. Additionally, increased ROS impair the mitochondrial function, damage mtDNA and accelerate the apoptosis of retinal capillary endothelial cells, pericytes and neurons [71,72]. Overexpression of mitochondrial superoxide dismutase (MnSOD) protects against the damage of retinal mtDNA and prevents development of diabetic retinopathy [70].

Moreover, modifications of the physical and biological properties of amino acids (such as cysteine, histidine, and tryptophan) in the eye lens may occur under the influence of phototoxic processes [73].

There is a balance between pro- and antioxidant reactions in the proper homeostasis of the retinal cell. However, the prolonged hyperglycemia in DM, increases hypoxia and advanced glycation ROS production as well as enlarges a variety of intracellular changes, such as overproduction of free radicals, mitochondrial dysfunction, and induces endoplasmic reticulum

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stress, leading to apoptosis [58]. Incorrect redox homeostasis, leading to neuronal cell loss, vascular abnormalities, and the development of late-stage diabetic retinopathy [74].

Hypoxia and hyperglycemia, the abnormal polyol and hexosamine pathway an increased diacyloglycerol level, nonenzymatic glycation of proteins with its endproducts, protein kinase C activation lead to a series of pathological events, including overproduction of ROS, oxidative stress, activation of proangiogenic factors and finally, inflammation with leukostasis.

- Inflammation with Leukostasis

Diabetic retinopathy is a disease of the retinal neurovascular unit, which refers to the functional coupling and interdependency of neurons, glia, and vasculature. Inflammatory responses play a prominent and complex role in propagating pathways further in diabetic retinopathy through cytokines, adhesion molecules, VEGF signaling, enhanced RAGE expression, changes in nitric oxide regulation and NF-κB signaling [75]. Leukocytes are characterized with large cell volumes, high cytoplasmic rigidity, and a natural tendency to adhere to the vascular endothelium via cellular adhesion molecules. This attachment, under hyperglycemic condition, could result in non-perfusion and ultimately proliferative diabetic retinopathy. Moreover, inflammation in the retina leads to increased intraocular blood pressure, formation of new weak vessels, and their increased permeability, resulting in hemorrhages in the retina and leukostasis. Leukostasis is manifested with clustering of neutrophils in the microcirculation. This is a significant factor in diabetic retinopathy, which is a progressive microvascular disease. Moreover, leukostasis is an early inflammatory response in DR, leading to damaging effects on the retinal circulating leukocytes and vascular endothelium [76].

The increase in blood glucose/dyslipidemia parallels the increased leukocyte trapping/leukostasis. It is associated with pathologically reduced capillary density and acellular capillaries in the later stages of diabetic retinopathy. Hyperglycemia activates circulating leukocytes, which are then prone to adhere to vascular walls. This leads to a progression from occasional transient leukocyte trapping to multiple sites of prolonged leukostasis with fewer successful reperfusions until networks of capillaries are permanently occluded in DR [77].

Kaji Y. and co-authors [78] reported that diabetic non-transgenic mice exhibit a threefold increase in the number of adherent leukocytes compared to non-diabetic control mice. Similarly, leukostasis increases in diabetic retinopathy. The number of adherent leukocytes is twofold higher in diabetic RAGE-transgenic mice compared to non-diabetic ones, even under normoglycemic conditions. However, systemic application of sRAGE significantly reduces the number of adherent leukocytes in the retinas of diabetic non-transgenic and diabetic RAGE-transgenic mice. Adherent leukocytes are detected on arterioles, venules and capillaries in the retinal vasculature.

Inflammatory cytokines, chemokines and growth factors such as TNF-α, IL-1β, hepatocyte growth factor, insulin-like growth factor-1, IL-6, MCP-1 and histamine promote leukostasis in DR. Moreover, the increased expression of inflammatory factors, including cell adhesion molecules, such as GMP-140, ICAM-1, CD11/CD18, and VCAM-1 are involved in inflammation processes with leukostasis. Leukocytes migrate through the endothelium into the extravascular space, where they differentiate into activated macrophages and secrete various cytokines and inflammation-related factors. Antibodies against CD-18 or ICAM-1, or genetic knockout of these genes in animals, can inhibit the leukostasis and decreased BRB breakdown [76]. Neutrophils of diabetic animals exhibit higher levels of surface integrin expression, such as CD18 and integrin-mediated adhesion. These mediators disrupt the cell–cell junctions, resulting in BRB breakdown. Long-term HG level intensifies expression of chemokines, e. g. CCL2, that enhance leukostasis, diapedesis and influx of monocytes into the retina and extravascular space [76].

Studies have also shown that leukostasis is a parameter that could help predict the severity of retinal vascular lesions in proliferative DR [79].

- Non-Coding RNAs as Biomarkers in DR

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Recently, non-coding RNAs (ncRNAs), including microRNAs (miRNAs), lncRNAs, and circRNAs, have appeared to be a specific biomarker for the early diagnosis and monitoring of diabetic retinopathy and also as optimistic therapeutic factors the disease treatment [80].

MicroRNAs are ribonucleic acids belonging to a family of small non-coding RNAs, located within introns and exons of protein-coding genes or in intergenic regions. MicroRNAs are participated in gene silencing by inhibiting protein synthesis through incomplete binding to the untranslated region of the target mRNAs, contributing to mRNA degradation. MiRNAs are engaged in the regulation of gene expression and they also play a key role in biological processes, such as cell proliferation, differentiation, growth control, angiogenesis, organogenesis, and apoptosis. [81]. Moreover, they are also involved in the occurrence and development of various diseases, including DR.

Cells release miRNAs into the bloodstream, where they viable for approximately 2 weeks. Due to their stability in plasma/serum/urine on freeze-thawing, efficient recovery and availability of quantitative detection methods make them a specific biomarker. Furthermore, the use of circulating miRNAs as biomarkers allows for non-invasive testing, which reduces patient discomfort and may increase patient compliance. Studies conducted recently have demonstrated impairment of the regulation of miRNAs in DR. A finding of new specific miRNAs as prognostic indicators for DR facilitates early detection of the diabetic retinopathy and allows to constantly monitor its development. Examination how microRNAs are involved in DR development could lead to better understand the disease itself and enable new treatments.

Some of exosomal microRNAs are engaged in the regulation of processes such as neovascularization, angiogenesis, apoptosis as well as inflammatory, neurodegenerative process and oxidative stress, which are involved in the pathomechanism of DR.

The latest studies have shown that abnormality regulation of microRNAs is related with the progression of DR, especially retinal cell dysfunction. For instance, five miRNAs (hsa-miR-195-5p, hsa-miR-20a-5p, hsa-miR-20b-5p, hsa-miR-27b-3p, and hsa-miR-451a) have been validated as biomarkers for stratification of DR stages [82], while miRNAs such as let-7a-5p and miR-211 have been shown to be upregulated in DR and related with expanded proliferation of retinal microvascular endothelial cells and suppression of expression of downstream target gene *SIRT1*, respectively [83]. Additionally, it has been observed that the miR-21 level is increased in DR and potentially induces angiogenesis via targeting PTEN (phosphatase and tensin homologue), leading to activation of AKT and ERK1/2 signaling pathways, and thereby enhancing HIF-1α and VEGF expression [84]. On the other hand, suppression of miR-126 in DR as against NPDR T2DM patients may be connected to endothelial impairment as it protects vascular endothelial cells and it as well makes possible vascular endothelial growth factor- A (VEGF-A) signaling [85].

Zampetaki et al. [86] conducted extensive research that represents an important advance in the field of miRNA biomarkers for DR. The authors performed a comprehensive analysis of miRNA expression profiles in DR using samples from two DR-related clinical trials on patients with T1DM: PROTECT-1 - with nonproliferative retinopathy and PREVENT-1 -without retinopathy. They identified 29 miRNAs that were differentially expressed between DR patients and controls. They included miR-27b-3p and miR-320a-3p, which were significantly associated with a high risk of DR. Furthermore, the authors used cultured human endothelial cells to investigate the potential mechanism underlying these miRNAs effects and identified thrombospondin-1 as a common target of miR-27b-3p and miR-320a-3p. These results suggest that the above miRNAs may engage in the development of DR. However, it is important to note that the study used a candidate miRNA approach, which may not be suitable to identify the best signature for DR due to the ability of a single miRNA to target multiple gene transcripts. Nonetheless, this study provides valuable insights into dysregulated miRNAs in DR and highlights a need for further research to validate these findings and explore the potential use of miRNAs as therapeutic targets or agents in treatment of retinopathy in diabetics.

Santovito D. et al. [87] found higher expression of miR-25-3p and miR-320b (another member of the miR-320 family), whereas changes in miR-320a-3p appeared to be statistically insignificant, as in

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Zampetaki's [86] study. The authors pointed out miRNAs identified in their experiment are enriched in other cell types as fibroblasts, stem cells and leukocytes, which might make them different.

Additionally, miR-23a, miR-320a and miR-320b have elevated expression in vitreous humor in patients with proliferative retinopathy versus subjects with macular hole (MH) [88], while miR-204 and let-7c were significantly downregulated in PDR patients in comparison to MH patients [89].

In contrast, specific miRNAs, such as miR-21, miR-181c, and miR-1179 [90], miR-93 [91], miR-221 [92] are potential biomarkers for diabetic retinopathy that enable to diagnose, prognosticate DR and also monitor its progression. Moreover, is the study revealed that miRNA-181c might be associated with vascular proliferation in high glucose (HG) [90].

In addition, miRNAs have as appeared to be important regulatory molecules in the pathogenesis of DR. A study by Wang et al. [93] showed that miR-409-5p might play a key role in neovascularization in DR. The authors investigated the expression of miR-409-5p in diabetic retinal tissues, mouse retinal microvascular endothelial cells and vitreous fluid of proliferative DR patients. The experiment explored the effect of miR-409-5p on retinal neovascularization in vitro and in vivo and aimed to provide a novel insight into the regulation pattern of miR-409-5p on DR. Knockdown of miR-409-5p suppresses VEGF-induced retinal neovascularization in vitro, while overexpression of miR-409-5p promotes proliferation, migration, tube formation and increases VEGF expression and secretion. PPARα is a downstream target of miR-409-5p, and its overexpression negates the promotion of miR-409-5p overexpression on the proliferation, migration, and tube formation of retinal microvascular endothelial cells (mRMECs). Knockdown of miR-409-5p attenuates retinal neovascularization in vivo, reduces the number of acellular capillaries per mm2 and the levels of neovasculogenic factors, including VEGF, TNF-α, ICAM-1 and MCP-1 in diabetic retinal tissues. The expression of miR-409-5p in vitreous fluid of proliferative DR patients was higher than that of control patients, and miR-409-5p was markedly increased in retinal tissues of STZ-induced DM mice and db/db mice in comparison with control mice. These findings suggest that miR-409-5p may be a key miRNA associated with neovascularization in DR and that anti-miR-409-5p therapy may represent a novel therapeutic strategy for DR in the future.

Additionally, the relationship between miRNA expression and oxidative stress signaling pathways in DR is an interesting topic of active research.

miRNA has been shown to modulate the expression of numerous gene transcripts in DR, which implicates its role in antioxidant protect of plant extracts and flavonol compounds.

For instance, high glucose and vitamin D treatment in human retinal endothelial cells can reduce miR-93 transcript, ROS production, MDA content, and elevated GSH activity [94]. miR-93 has also been shown to target SIRT1 in rat of streptozotocin-induced diabetes, contributing hereby to changes indicative of oxidative stress and inflammation which can be reversed by SIRT1 overexpression [95]. Another miRNA, miR-338-3p, negatively regulates glutamine transporter SLC1A5 expression leading to ferroptosis [96]. In addition, inhibition of mitochondrial SIRT transcripts: SIRT3, SIRT4 and SIRT5 by miR-1, miR-19a, and miR-320a respectively are implicated in oxidative damage in diabetic retinopathy [97].

Notably, miRNA-145 overexpression can reduce ROS and malondialdehyde (MDA) levels, increase superoxide dismutase activity and decrease HG-induced oxidative stress and retinal endothelial cell apoptosis [98]. Moreover, miR-183 stimulates the PI3K/Akt/VEGF signaling pathway, raises CD34, eNOS, and free radicals [99]. On the other hand, miR-27 suppresses the Nox 2 signaling pathway by down-regulating P13K/AKT/mTOR, thus decreasing ROS concentration [100].

The activation of the polyol pathway by hyperglycemia increases the activity of aldose reductase, which in turn, decreases expression of miR-200a and miR-141 (these miRNAs are regulators of KEAP-1 (Kelch-like ECH-associated protein 1)), suppressing NRF2 (nuclear factor erythroid 2-related factor 2) and resulting in increases ROS and oxidative stress. However, aldose reductase deficiency in the renal cortex upregulates miR-200 and miR-141, releases the KEAP-1 suppression of NRF2 and ameliorates oxidative stress, preventing kidney fibrosis [101].

The second group of RNA molecules belonging to the family of non-coding RNAs and increasingly studied are long non-coding RNAs (lncRNAs). LncRNAs are RNA molecules that do not

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have significant protein-coding potential and are over 200 nucleotides long, making them a distinct class of transcripts. More than 100,000 lncRNA genes have been identified in humans. They can be involved in multiple biological processes through different molecular mechanisms and play a vital role in various biological processes, such as post-transcriptional gene regulation and epigenetic gene silencing [102]. LncRNAs have versatile and critical roles in various diseases, such as cancers [103], neurodegenerative diseases [104], diabetes, and complications such as diabetic retinopathy [105].

LncRNAs can regulate gene expression in both cis and trans regulatory relationships and form regulatory networks with coding mRNAs. LncRNAs can be classified as nuclear-retained or cytoplasmic. Introns exist in both protein-coding and non-protein-coding genes are the main origin of regulatory noncoding RNAs, including lncRNAs [102]. Nuclear-retained lncRNAs play important roles in transcriptional regulation, while one of key roles of cytoplasmic lncRNAs in gene expression regulation is played through miRNA sponge activity, where lncRNAs can act as competing endogenous RNAs (ceRNAs), regulating mRNA expression by sequestering miRNAs [106]. Furthermore, lncRNAs have unique expression patterns and were most often found to be upregulated in different contexts related to DR.

XIST (X Inactive Specific Transcript) is a widely studied lncRNA. It has been found to be downregulated in high glucose-treated Müller retinal cells separated from a diabetic mouse and human Müller retinal cell line and ARPE-19. XIST has been found to exert its functions by sponging miRNAs, and its upregulation causes miR-21-5p suppression, which could affect the regulation of VEGF signaling. Over-expression of XIST has a protective result on migration and apoptosis in ARPE-19 treated with high glucose concentrations, also decreasing of pro-inflammatory cytokines in human Müller cells and in HG-treated mice [107,108].

MIAT (Myocardial Infarction Associated Transcript) is lncRNA whose potential protective role in DR has been identified. MIAT is located on the 22q12.1 locus linked with myocardial infarction susceptibility [109]. MIAT plays an important role in numerous biological processes such as neuronal survival and formation of nuclear bodies [110,111]. Li et al. [112] indicate that plasma tests from diabetic retinopathy subjects demonstrated upregulation of MIAT versus both groups without DR and the control group. Moreover, samples from animal diabetic retinas and endothelial cells cultured in high glucose medium showed upregulation of MIAT and involvement in regulating the endothelial cell function and pathological angiogenesis [113].

Another lncRNA, i.e., RNCR3 (Retinal Non-Coding RNA 3), has also been found to play a role in protecting against DR. This lncRNA has been linked to neuronal and oligodendrocyte differentiation and in atherosclerosis-related vascular impairment [114]. Knockdown of RNCR3 has been shown to alleviate retinal microvascular leakage and inhibit migration and tube formation by RF/6A cells under high glucose conditions. RNCR3 is upregulated in high glucose-treated in RF/6A cells and retinas of diabetic mice as well as in fibrovascular membranes of PDR patients. Inhibition of RNCR3 may therefore be a potential treatment option for preventing DR-related retinal anomalies [115]. Moreover, another study showed that RNCR3 knockdown significantly decreases retinal reactive gliosis [116]. Inhibition of RNCR3 leads to a significant reduction in the release of cytokines: IL-2, IL3, IL-4, IL-5, IL-9, IL-13, IL-17, MCP-1, VEGF and TNF-α. Moreover, treatment with intravitreal RNCR3 shRNA hinders glial cell reactivity and induces reductions in cytokines in diabetic mice. Furthermore, shRNA-mediated knockdown of RNCR3 reduces acellular capillaries and retinal vascular leakage in diabetic retinas. There is also a significant decrease in cytokines, such as MCP-1, TNF-α and VEGF-A in the retinas of diabetic mice following intravitreal RNCR3 shRNA administration. The experiment showed that RNCR3 knocks down the reduction in apoptotic retinal cells, prevents HG-induced retinal neurodegeneration and improves visual function. Overall, these findings suggest that lncRNAs such as RNCR3 play a crucial role in the protective mechanisms against DR and provide a potential therapeutic target for DR treatment [116].

Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1) is another lncRNA widely studied in DR. Studies have shown that dysregulation of MALAT1 expression plays a key role in the pathogenesis of diabetic retinopathy. Hypoxia, which is a central pathophysiological phenomenon in diabetes-targeted organs, induces the transactivation of the MALAT1 promoter

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through the enhanced activity of hypoxia-inducible factor-1α (HIF-1α) in vitro [117]. In the retina, MALAT1 can modulate, development of neurodegeneration by inducing the cAMP – a response element binding protein (CREB) and p38 MAPK signaling pathway. MALAT1 upregulation can dysregulate microvascular growth, even disturbing the activity of a retinal endothelial cell, while MALAT1 knockdown ameliorates DR in vivo and regulates endothelial cells functions (cell proliferation, migration, and tube formation) in vitro via cross-talk between MALAT1 and the p38 MAPK pathway [118].

Puthanveetil et al. [119] documented that MALAT1 knockdown in human umbilical vein endothelial cells (HUVECs), under hyperglycemic conditions, down-regulates serum amyloid antigen 3 (SAA3) activation, subsequently reducing the RNA and protein expressions of key inflammatory mediators (IL-6 and TNF-α) implicated in diabetic complications. Biswas et al. [120] made a particularly interesting observation by revealing that MALAT1 is able to impact expressions of inflammatory transcripts through its association with components of the Polycomb repressive complex 2 (PRC2) in diabetes. PRC2 is a chromatin-modifying enzyme that catalyses the methylation of histone H3 at lysine 27. Moreover, MALAT1, TNF-α and IL-6 expression is up-regulated in the vitreous humors from diabetic patients. Additionally, Radhakrishnan et al. [121] observed that siRNA-mediated knockdown of MALAT1 in human endothelial cells of the retina (HRECs) modulates antioxidant defense in DR. MALAT1 suppression conducts to down-regulation of inhibitor Keap1 and dissociation of NRF2, thus stimulating transcription of antioxidant and detoxifying genes.

The Antisense Non coding RNA in the INK4 Locus (ANRIL) expression dysregulation also has been identified as a key mechanism in the pathogenesis of diabetic retinopathy. ANRIL is known to regulate VEGF expression and function in DR. Thomas et al. [122] observed that ANRIL-knockout diabetic mice and ANRIL-silenced human retinal endothelial cells (HRECs) under HG stress show reduced levels of VEGF expression via binding p300, the enhancer of Zeste homolog 2 (EZH2) of the PRC2 complex and miR-200b. Knocking down ANRIL levels using siRNAs prevents VEGF upregulation at mRNA and protein levels, thereby inhibiting cell proliferation and tube formation. Furthermore, Toraih et al. [123] demonstrated that sample of DR patients shows upregulation of ANRIL, contrary to both groups without DR and control inividuals. Another study describes no differences in ANRIL expression in plasma of DM subjects versus control group, whereas the upregulation has been found in serum, aqueous humor, and vitreous humor of patients with NPDR and PDR, contrary to both DM subjects without DR and control participants [124].

In contrast, the low levels of SOX2 overlapping transcript (SOX2OT) is linked to neurodegenerative complication. In humans, SOX2OT is highly expressed in the brain, and may possibly be engaged in the regulation of retinal neural function, thereby impact on retinal neurodegeneration process [125]. Decreased expression of SOX2OT is reported in the retinas of STZ-induced diabetic mice as well as retinal ganglion cells under HG or oxidative stress and regulates NRF2/HO-1 signaling activity, hereby proving its antioxidant activity and neuroprotective function in diabetes-related retinal neurodegeneration in vivo. These findings suggest that SOX2OT knockdown may be a potential treatment benefits for diabetic retinopathy [126]. Additionally, mice with diabetic nephropathy compared to control mice, display decreased levels of SOX2-OT, and this result was confirmed in cultured human podocytes and mesangial cells [127].

Recent research has shown that lncRNAs have significant potential as disease-specific biomarkers for diagnosis of DR. In particular, two upregulated lncRNAs have demonstrated high sensitivity in distinguishing PDR patients from NPDR patients and the control group. These lncRNAs exhibit a disease state-specific pattern, making them good candidates for biomarker development. The high sensitivity and specificity of these lncRNAs in distinguishing PDR patients suggest that they could serve as potential biomarkers in prediction and diagnose of DR. Blood ENST00000505731 and NR-126161 lncRNAs, in particular, have shown a potential diagnostic value for DR. However, the authors explained that study is limited by its sample size, and future prospective screening studies on larger groups are necessary to increase the sensitivity of the assay and determine whether certain sub-stages of DR can be detected through lncRNA expression levels and/or combination of

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lncRNA phenotypes before clinical diagnosis. Overall, this study provides valuable insight into the potential apply of lncRNAs as medical indicators for DR, which could have implications for improving early detection and treatment of this debilitating disease [128]. Dysregulated lncRNAs involved in diabetic retinopathy are summarized in Table 1.

Table 1. Dysregulated lncRNAs involved in diabetic retinopathy.

lncRNAs	Sample	dysregulation	pathogenic effects	Reference
XIST	human retinal pigment epithelial ARPE-19 cells;	downregulated	apoptosis, migration	Dong Y. et al. 2020
	mouse retinal Müller cells (mMCs) human retinal Müller cell line (HMCs)	downregulated	inflammation	Zhang J. et al. 2021
MIAT	human plasma, ARPE-19 cells;	upregulated	decreases retinal pigment epithelial cells viability	Li Q. et al. 2018
	HMVECs, RF/6A, RPE, RGC and Müller cells	upregulated	angiogenesis, proliferation, migration, and survival of endothelial cells	Yan B. et al. 2015
RNCR3	retinas of DM mice, RF/6A cells	upregulated	retinal vascular functions; proliferation, migration, proinflammatory activation of ECs and VSMCs	Shan K. et al. 2016
	retinas of DM mice, Müller cells	upregulated	retinal vascular functions, release of several cytokines	Liu C. et al. 2016
MALAT1	retinas of DM mice, Müller cells, primary retinal ganglion cells (RGCs)	upregulated	retinal neurodegeneration	Yao J. et al. 2016
	human umbilical vein endothelial cells (HUVECs)	upregulated	inflammation	Puthanveetil P. et al. 2015
	human retinal endothelial cells (HRECs), diabetic mice;	upregulated	inflammation	Biswas S. et al. 2018

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	vitreous humor (VH): T2DM patients			
	human endothelial cells of the retina (HRECs)	upregulated	oxidative stress	Radhakrishnan et al. 2021
ANRIL	HRECs, diabetic mice	upregulated	regulates VEGF, proliferation, migration, tube formation, vascular permeability	Thomas et al. 2017
	blood serum, aqueous fluid and vitreous fluid: T2DM patients with NPDR and PDR vs. T2DM without DR	upregulated	-	Chen S. et al. 2019
	blood serum: T2DM patients vs. HC	no difference	upregulated VEGF, neovascularization, angiogenesis	Chen S. et al. 2019
	blood plasma from T2DM patients with DR/without DR	upregulated	no association with DR progression	Toraih E.A. et al. 2019
SOX2-OT	retinas of DM mice, RGCs	downregulated	retinal neurodegeneration	Li C-P. et al. 2017
ENST-00000505731 NR-126161	whole blood: T2DM patients with NPDR and PDR vs. HC	upregulated	can serve as diagnostic biomarkers to detect DR early and monitor its progression	Liu B. et al. 2022

CircRNAs (circular RNA) are classified as a subgroup of long non-coding RNAs. Yet, it has recently been reported that some of them may encode proteins [129,130]. It is a distinct type of RNA molecule characterized by a covalent bond linking the 3' and 5' ends, which results in a closed loop structure. Unlike linear RNA, circRNAs are formed by a non-canonical event called back-splicing, where the 3' end of an exon is covalently linked to the 5' end of another exon [131]. Their circular structure is resistant to RNase R - an exonuclease that degrades almost all linear forms of RNA. Due to this shape, it is a very stable molecule. They are characterized with specific expression and complex regulation, with tissue-specific or developmentally specific expression patterns. They regulate gene expression by acting as miRNA sponges. Some of them are believed to regulate the function of microRNAs (miRNAs) and play a role in transcriptional control. Moreover, circRNAs are able to bind to RNA-binding proteins (RBPs) and are involved in the regulation of translation [132]. Moreover, circRNAs can code small proteins and peptides. In comparison to their linear host gene products, circRNAs-encoded proteins or peptides perform independent biological functions. For example,

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circAKT3, which encodes a 174 amino acid novel protein, can negatively regulate the RTK/PI3K (Receptor Tyrosine Kinases/the phosphatidylinositol 3' -kinase) pathway [133].

Most circular transcripts are tissue-specific. CircRNA accumulation is higher in slowly dividing cells, e.g. in the brain, compared to rapidly dividing cells, e.g. in the liver [134]. High accumulation of circRNA is also found in peripheral blood (especially in erythrocytes and platelets), in healthy organs, cancerous tumors and cell lines as well as in body fluids, such as urine and saliva. Large amounts of circRNA have been detected in exosomes [135,136].

Due to the fact that circRNAs are characterized by high stability and specificity depending on the type of cells or various pathological conditions, it makes them good potential biomarkers. Therefore, circRNAs have become a new research hotspot in the field of RNA, with thousands of human circRNAs being identified using molecular biology and bioinformatics methods.

Zhang SJ et al. [137] found that one of the circular RNAs derived from the HAS2 gene locus, circ_0005015, was significantly upregulated in diabetic retinas, vitreous samples, plasma fractions of whole blood and preretinal fibrovascular membranes (FVMs) of DR patients. Functional assays have revealed that circ_0005015 can regulate the retinal endothelial cell function by acting as a miRNA sponge, and have been proven to play a vital role in the progression of DR by regulating the growth, proliferation, migration and tube formation of retinal vascular endothelial cells. CircRNA homeodomain-interacting protein kinase 3 (circHIPK3) and circRNA zinc finger protein (cZNF609) also play a similar role [138,139]. CircRNA zinc finger protein 532 (circZNF532), produced in the cytoplasm of pericytes is another such molecule. CircZNF532 regulates the expression of NG2, LOXL2 and CDK2 proteins, which are essential in maintaining vascular stability. Notably, overexpression of circZNF532 decreases the diabetic consequence on microangiopathy, protecting against diabetes-induced retinal pericyte degradation and vascular damage, highlighting its significance in DR pathogenesis. Moreover, circZNF532 acts as an miR-29a-3p sponge to regulate gene expression, making it a potential therapeutic target in DR treatment [140]. In contrast, Wang et al. [141] revealed that circZNF532 contributed to expedited high glucose concentrations-induced human retinal microvascular endothelial cells (hRMEC) dysfunction via sponging miR-1243 and activating arginine methyltransferase 1 (CARM1).

Hypoxia-induced circRNA euchromatic histone lysine methyltransferase 1 (circEhmt1) in the nucleus of pericytes regulates pericyte-endotheliocyte crosstalk, which plays an important role in the pathogenesis of DR [142]. Another circRNA PWWP domain, containing 2A (circPWWP2A), acts as an miR-579 sponge and upregulates the angiopoietin-1/Occluding/Sirtuin 1 proteins, thus contributing to the promotion of DR progression [143]. Dysregulated circRNAs involved in DR are summarized in Table 2.

Table 2. Dysregulated circRNAs involved in diabetic retinopathy.

circRNAs	Sample	dysregulation	pathogenic effects	Reference
circ_0005015	diabetic retinas, vitreous samples, plasma fractions of whole blood, preretinal fibrovascular membranes (FVMs) of DR patients	upregulated	promote the growth, proliferation, migration, and tube formation of retinal vascular endothelial cells	Zhang SJ et al. 2017
circHIPK3	retinas of DM mice	upregulated	endothelial proliferation, migration, angiogenesis, inflammation, vascular dysfunction	Shan, K et al. 2017

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circZNF609	HUVECs, retinas of DM mice, Müller cells	upregulated	migration, tube formation, angiogenesis, apoptosis, oxidative stress	Liu C et al. 2017
circZNF532	human retinal pericytes (ACBRI-183) and HRVECs retinas of DM mice vitreous humor of DM patients	upregulated	pericyte degeneration, cell viability, angiogenesis, breakdown of BRB	Jiang, Q et al. 2020
	blood serum: T2DM patients vs. HC human retinal microvascular endothelial cells (hRMECs)	upregulated	proliferation, migration, angiogenesis, inflammation	Wang T et al. 2022
circEhmt1	mice, retinal microvascular pericytes and endotheliocytes	downregulated	migration, tube formation, angiogenesis, apoptosis, neovascularization, inflammation	Ye, L et al. 2021
circPWWP2A	DM mice, human retinal microvascular endothelial cells (HRVECs), human retinal pericytes	downregulated	retinal microvascular leakage, angiogenesis, apoptosis, neovascularization	Liu C et al. 2019

Conclusions

Diabetic retinopathy is a complex microvascular disease characterized by various clinical manifestations and biochemical pathways that contribute to its pathophysiology. The exact mechanism responsible for the development and progression of DR is not fully understood, but extensive research has revealed several key molecular processes involved in this disease. Hyperglycemia plays a significant role in the pathogenesis of DR, triggering a cascade of events that lead to oxidative stress and vascular damage. The formation of ROS due to elevated glucose levels results in retinal oxidative stress, DNA metabolism and the activation of PARP) and NF-κB signaling pathways. Increased oxidative stress causes a deficit of pericyte and neuronal cells, blocked capillaries and distortion of the microvascular structure of the retina. Furthermore, the damage of pericytes is caused by increased activity of protein kinase C-delta signaling induced by raised glucose concentrations. The breakdown of the blood-retina barrier is a crucial event in the pathophysiology of DR, leading to vascular leakage and edema in retinal tissues. Oxidative stress also contributes to early evidences of diabetic retinopathy, including a thickened basement membrane, mitochondrial dysfunction and pericyte apoptosis, which further exacerbate the blood-retina barrier extenuation. These molecular processes and pathways interplay in a complex manner, resulting in occurrence of characteristic clinical features of diabetic retinopathy, such as elevated vascular flow, vascular lesions, inflammation neovascularization and neurodegeneration. Understanding these mechanisms

is essential for designing effective treatment modalities that can prevent or slow down the progression of diabetic retinopathy, as it is a substantial burden to individuals and socio-economy. Furthermore, studies have shown that diabetes can influence circulating non-coding RNAs expression, with some it serving as diagnostic indicators for DR diagnosis. However, more research is needed to properly investigate the mechanisms underlying non-coding RNAs (miRNAs, lncRNAs, circRNAs) involvement in DR and identify a non-invasive diagnostic and prognostic ncRNA signature of DR, as well as to explore distinct pathways and ncRNAs that underlie the development of DR in Type 1 or Type 2 diabetes. More advanced investigations are needed to develop targeted therapies with fewer side effects for this sight-threatening disease.

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