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MicroRNAs In Pathogenesis of Diabetic Retinopathy

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ABSTRACT

A novel non-transmissible pandemic obesity has escalated the incidence rate of diabetes in present world population. Both T1D and T2D are associated with considerably accelerated rates of multiple microvascular as well as macrovascular complications. Diabetic retinopathy is one of the most debilitating chronic impediments, although the exact mechanism(s) responsible for how retina is damaged in diabetes remains unclear. Although the key risk factors contributing to these complications like hyperglycemia, hyperlipidemia, advanced glycation end products, growth factors and inflammatory molecules are well-identified, the currently available therapies are not completely efficacious. Therefore there is an imperative requirement for a better elucidation of the molecular machinery underlying the high incidence of diabetic complications in order to identify newer therapeutic and prognostic markers. MicroRNAs are short non-coding RNAs that can repress target gene expression via post-transcriptional mechanisms and involved in a number of biological processes, including the pathogenesis of diseases. Easily detectable and consistent alterations in microRNA levels in body fluids of the patients due to hyperglycemia-induced epigenetic alterations of metabolic events make them an attractive candidate for early diagnosis prior to clinical manifestation of disease. Also, a number of such microRNAs exhibit therapeutic potential enhancing the provision for improved management of diabetes and associated complications like DR. Herein, the role of microRNAs in the pathobiology of diabetic retinopathy has been elaborated considering their possible utilization as biomarkers and therapeutic targets.

Keywords: diabetes, hyperglycemia, diabetic retinopathy, microRNA, biomarker, therapeutic

Diabetes is a chronic metabolic disorder with increasing prevalence globally and is estimated to rise to 552 million adults by 2030[1]. Diabetes results in elevated fasting blood sugar levels either due to the inability to produce adequate insulin or insulin resistance. While type 1 diabetes (T1D) is characterized by the destruction of pancreatic beta cells and loss of insulin production, type 2 diabetes (T2D) involves progressive insulin resistance and beta cell dysfunction[2,3].

More than 6% of the world's population is affected by T2D and the incidence across the world is projected to be twofold by 2025[4]. Type 2 diabetes mellitus (T2DM) is largely defined by hyperglycemia which promotes microvascular as well as macrovascular complications in the pathological inscription of the disease[5]. The macrovascular complications include cerebrovascular disease, coronary heart disease, and peripheral vascular diseases. Pathological changes in the diabetic microvasculature can alter organ perfusion, particularly affecting organs like retina, kidney and peripheral nervous system that are profoundly dependent on their microvasculature supply[6,7]. A large burden of T2DM morbidity is driven by clinical problems associated with these alterations, namely, diabetic retinopathy, nephropathy, and neuropathy[7].

Diabetic retinopathy: A microvascular complication

Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes leading to loss of vision and even blindness if left untreated[8,9]. It affects up to 80% of all patients who have had diabetes for 10 years or more [10]. Diabetic macular edema (DME) accounts for 75% of DR-related vision loss while impediments of proliferative diabetic retinopathy (PDR) being accountable for majority of the rest[9]. In different epidemiological studies the prevalence of DR ranges from 15.3 to 42.4% and both modifiable risk factors (hyperglycemia, hypertension, hyperlipidemia, and smoking) and non-modifiable risk factors (duration, age and genetic predisposition) are responsible for progression of the disease[10-12].

Major symptoms include pericyte death and thickening of the basement membrane, leading to weak vascular walls. The blood-retinal barrier (BRB), which comprises the retinal vasculature and the retinal pigment epithelium (RPE), excludes the neural elements of the retina and the cytotoxic products from circulating inflammatory cells, thereby protecting the retina and allowing it to selectively regulate its extracellular chemical composition[13]. DR results from abnormal retinal blood vessels that are either non-proliferative or proliferative. Hyperglycemia can result in increased inflammatory responses and vascular dysfunction which in turn lead to retinal microvascular flaws, neuro-retinal dysfunction and degeneration[14,15].

It has also been suggested that in the pathogenesis of diabetes, sustained hyperglycemia triggers apoptosis in RPE cells, thereby contributing to the progression of diabetic retinopathy[16,17]. Induction of reactive oxygen species (ROS) generation is causally linked to high glucose-mediated toxicity to different types of cells[18,19]. The resultant damages are pivotal to the BRB imbalance that leads to the leakage of fluids and lipids into the retina contributing to DR progression[20].

Diabetic retinopathy: Causes and consequences

The alarming rate of increasing mortality and morbidity associated with DR incurs considerable economic liability related to both treatment and social support to the patients[21,22]. Preliminary management of the disease by tight glycemic and blood pressure control is becoming dubious due to increased caloric consumption and the resultant obesity epidemic. The disease remains asymptomatic until the pathology is significantly advanced[23]. The currently available treatments for DR are applicable only at advanced stages of the disease including laser photocoagulation, steroids, or antivascular endothelial growth factor (VEGF) injections and are associated with significant adverse effects[24-26]. Therefore, it is of vital importance to develop additional novel therapeutics for diabetic retinopathy as well as biomarkers to enable early detection of the disease so as to control the risk factors tightly in the initial stage.

Epigenetics in Diabetic retinopathy: In search of a biomarker

A biomarker has been defined as "a biological molecule found in blood, or other bodily fluids, or tissue which represents a sign of a normal or abnormal process of a condition or disease"[27,28]. Biomarkers may help to identify people with subclinical disease as well as to monitor various aspects of the clinical disease like response to treatment[29]. As the retina constitutes a small proportion of total body weight, a circulating biomarker for DR needs to be highly specific compared to a systemic one. Biomarkers encompass a repertoire of biomolecules ranging from glycation end products, components of extracellular matrix, immuno-complexes, autoantibodies to different circulating nucleic acids. Nucleic acids are identifiable in peripheral blood, so providing a new potential tool for diagnosis and/or prognosis.

Development of a large number of diseases, coordinated through differential gene expressions cannot be explained solely by changes in nucleotide composition of genome, rather other heritable modifications like DNA methylation, histone acetylation, non-coding RNA regulation, and chromatin remodeling also play an intrinsic role in the onset of diseases. Diabetic environment

disturbs metabolic homeostasis and also alters various genes, including genes associated with oxidative stress, apoptosis and inflammation[30-32]. The vital mechanisms of epigenetic modifications are reported to be involved in the "metabolic memory phenomenon" as a result of prolonged exposure to hyperglycemia leading to progression of DR despite of well-controlled blood glucose in advanced diabetic patients[33,34]. Moreover the reversible nature of epigenetic changes may provide new insights for the treatment of such diseases[33]. This review emphasizes that reversibility of microRNA expression could provide a new strategy for the prevention and treatment of diseases.

MicroRNAs and Diabetic retinopathy

MicroRNAs are endogenously produced short coding RNAs of ~20–22 bp length that have an important role in modulating gene expression, inhibiting the expression of their target genes by posttranscriptional modifications[35,36]. MicroRNAs are considered as potential biomarkers for the early detection of DR due to their stability in biofluids, easy quantitative estimation, and because some of them are cell-type or tissue-specific[37,38]. In recent past, several microRNAs have emerged as important regulators of DR pathobiology[39].

One of the first studies demonstrating the significance of microRNAs in DR was performed by Kovacs et al. An elaborate microRNA-expression profiling assay was conducted on the retina and retinal endothelial cells of streptozotocin-induced diabetic rats leading to the identification of differential expression of several microRNAs marked for the overexpression of nuclear factor (NF)-kB, vascular endothelial growth factor (VEGF), and p53. Upregulation of these transcription factors are characteristic signature of the pathologic state of retinopathy. Among the differentially expressed microRNAs, most notable are microRNAs such as miR-146, miR-155, miR-132 and miR-21that are particularly (NF)- k B-responsive. MiR-146, in particular, was identified as a potential therapeutic target, through its negative feedback regulation of NF-k B activation in retinal endothelial cells[40]. In a more recent study, Feng *et al.* demonstrated downregulation of miR-146a in hyperglycemia-induced endothelial cells from large vessels and retinal microvessels and in retinas from T1D rats. Fibronectin, a mediator of hypertrophy and fibrosis, was identified to be a direct target of miR-146a, suggesting that miR-146a down-regulation and simultaneous upregulation of p300 could be a crucial strategy for elevated production of extracellular matrix protein in diabetes. Moreover, intravitreal inoculation of miR-146a mimics reinstated retinal miR-146a reducing fibronectin levels in diabetes suggesting possible development of such translational approaches for DR treatment[41].

Activation of pro-inflammatory signaling is a key pathological response of diabetic retinopathy contributing to endothelial dysfunction. Some recent reports indicated that miR-15a/16 played a role in reducing pro-inflammatory signaling of IL-1β, TNFα, and the phosphorylation of NF-κBp65 (ser536) in cultured human retinal endothelial cells, which have already been identified to associated with pathogenesis of early DR[42,43].

In the study of Kovacs *et al.* members of miR-34 family responsive to p53 were also implicated to be upregulated in diabetic rats and some of them are important markers in the retina[40]. In another study miR-34a was reported to be a key player in suppressing the proliferation and migration of retinal pigment epithelial (RPE) cells through down regulation of its targets c-Met and other cell cycle-related molecules which is crucial in proliferative vitreoretinopathy (PVR)[44]. In a similar mechanism miR-182 was also observed to inhibit HGF/SF-induced increases in RPE proliferation and migration in PVR[45].

A report by Silva *et al.* found that both miR-29b and its potential target RAX (an activator of the pro-apoptotic PKR signaling pathway), were delimited in the retinal ganglion cells and the cells of the inner nuclear layer of the retinas from streptozotocin diabetic rats and control rats. The authors advocated that overexpression of miR-29b during in preliminary stages of diabetes may be protective against apoptosis of retinal neurons through the PKR pathway[46].

A microarray-based study revealed differential expression of 304 microRNAs in the TGFβ2-induced epithelial-mesenchymal transition of human retinal pigment epithelium cells of both proliferative vitreoretinopathy (PVR) and proliferative diabetic retinopathy (PDR). This observation can be imperative in the identification of microRNAs associated with PVR and PDR progression, and their utilization as potential therapeutic targets for these diseases[47].

Endothelial cell damage is considered to be one of the major mechanisms triggering retinal microvascular injury in diabetes. In a recent study Mortuza et al identified that hyperglycemia-induced overexpression of miR-195 is instrumental in regulating sirtuin (SIRT)-1 mediated tissue damage in DR. The expression of miR-195 was elevated in retinas of diabetic rats whereas intravitreal injection of antagomiR-195 amended levels of SIRT-1[48]. Another microRNA microarray based study has identified a novel mechanism whereby miR-23b-3p negatively regulates high-glucose-induced cellular metabolic memory in DR through a SIRT-1-dependent signaling pathway by inhibition of Ac-NF-κB expression[49].

Furthermore, hypoxia-inducible factor 1 alpha (HIF1α) and VEGF are both implicated in the pathogenesis of DR[50-52]. Lately Ling *et al.* observed a repartee between HIF1and VEGF through the expression of twelve common microRNAs and that silencing either HIF1or VEGF increased the availabilities of the shared miRNAs. Particularly, over-expression of miR-106a significantly reduced the expression of HIF1α and VEGF and prevented high glucose-induced increased permeability[51]. Similarly, miR-351-dependent crosstalk between Ang-2 and VEGF was found to play a role in hypoxia-induced microvascular response characteristic of diabetic retinopathy[52]. In addition, miR-126 expression was shown to remain suppressed under hypoxic conditions in vivo and in vitro, but overexpression of the microRNA can be engineered to halt the hypoxia-induce neovascularization by suspending the cell cycle progression through modulation of VEGF and MMP-9 protein expression in monkey chorioretinal vessel endothelial cells (RF/6A)[53].

Moreover, upregulation of miR-200b has been involved in the controlling the expression of oxidation resistance (Oxr)-1, a protein controlling the sensitivity of neuronal cells to oxidative stress, in the retinas of Akita mice, a model of type 1 diabetes, suggesting a protective role of the microRNA in DR[54]. At the same time in another study the same microRNA was reported to regulate VEGF-mediated alterations in diabetic retinopathy both in vivo and *in vitro*[55]. Since, anti-VEGF therapies are being appraised for DR, it is worth evaluating microRNAs that are known to target VEGF.

These findings could be very significant from a therapeutic perspective, since miRNAs are important in neovascularization, matrix protein accumulation and vascular permeability, all important contributors for loss of vision. Moreover, considering the success of intravitreal delivery approaches for retinal disorders, treatments with mimics of "protective" microRNAs like miR-146a, miR-29b and miR-200b could be developed as treatment modalities for DR. However, more extensive in vivo research work is essential to identify and validate the specific targets and pathways that can be modulated by some of these differentially expressed biomarkers.

CONCLUSION

In this review work, the role of microRNAs in diabetic retinopathy has been discussed elaborately considering their differential expression and complex signaling. MicroRNAs are fascinating zone of RNA biology due to their roles in the orchestration of many physiological processes, as well as pathophysiological disease state. MicroRNAs are regulatory molecules that contribute to numerous aspects and phases of diabetes and its complications by triggering specific signaling pathways, altering the expression of certain genes. Therefore, some of these molecules may provide valuable information within a clinical background, either as screening tools for high-risk patients, or as early prognostic tools, and thus influencing the procedure of treatment decision-making. The effects of these microRNAs depend upon cell-type specific patterns, the type

of model systems employed, time of sampling and the severity of the complications in the models studied. Furthermore, since a single microRNA can potentially target multiple genes, it is challenging to develop microRNA-specific therapeutics due to probable off-target effects. Therefore, many of these markers still need to be validated in *in vivo* scenario where the complexity and diversity of interactions may overturn the attempt for clinical applications. Nevertheless, once authenticated *in vivo*, they may themselves be considered direct therapeutic targets.

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