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Highlights

- Dismetabolic pathways in diabetes mellitus (DM) involve the Sirtuins (SIRTs)
- SIRTs are a recently discovered class of 7 histone deacetylases altered in DM
- Retinopathy in DM, inflammation and oxidative stress are strictly related to SIRTs
- SIRTs activators may be employed as novel therapeutic approaches in DM
- Clinical trials will be needed to test new molecules modulating the SIRTs activity

Diabetic retinopathy, oxidative stress and sirtuins: an in depth look in enzymatic patterns and new therapeutic horizons.

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Abbreviations

Adenine dinucleotide phosphate (ADP-ribose)
Adenosine monophosphate (AMP)
Adenosine monophosphate-activated protein kinase (AMPK)
Advanced glycation end products (AGEs)
AMPK activator 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR)
Blood-retinal barrier (BRB)
Brain derived neurotrophic factor (BDNF)
Breakdown of the inner blood-retinal barrier (BBRB)
Cyclooxygenase-2 (COX-2)
Diabetes mellitus (DM)
Diabetic retinopathy (DR)
Endothelial nitric oxide synthase (eNOS)
Endothelin-1 (ET-1)
Ergothioneine (EGT)
Exendin-4 (EX-4)
Fibronectin (FN)
Forkhead box O1 (FOXO1)
Ganglion cell layer (GCL)
Glial acidic fibrillary protein (GFAP)
Glucagon-like peptide-1 (GLP-1) analogue
High mobility group box 1 (HMGB1)
Histone deacetylases (HDACs)
Hypoxia-induced factor-1α (HIF-1α)
Human retinal endothelial cells (HRECs)
Human retinal pigment epithelial cells (ARPE-19)
Human umbilical vein endothelial cells (HUVECs)
Inducible nitric oxide synthase (iNOS)
Intercellular adhesion molecule-1 (ICAM-1)
Internal plexiform layer (IPL)
Interleukin (IL)
Liver x receptor (LXR)
Manganese superoxide dismutase (MnSOD)
Maternally expressed gene 3 (MEG3)
Metalloproteinase-9 (MMP-9)
MicroRNA (miRNA)
Nicotinamide adenine dinucleotide (NAD+)
Nicotinamide adenine dinucleotide phosphate (NADPH)
Nicotinamide adenine dinucleotide phosphate oxidase (NOX)
Nicotinamide mononucleotide adenyltrasferase 1 (NMNAT1)
Nitric oxide (NO)
Nitric oxide synthase (NOS)
Nonalcoholic fatty liver disease (NAFLD)
Non proliferative diabetic retinopathy (NPDR)
N-[2-hydroxy-3-(1-piperidinyl) propoxy]-3-pyridinecarboximidamide, dihydrochloride (BGP-15)
Nuclear factor-κB (NF-κB)
Optical coherence tomography (OCT)
Peripheral blood mononuclear cells (PBMCs)

Peroxisome proliferator-activated receptor-α (PPAR-α)
Peroxisome proliferator-activated receptor gamma coactivator (PGC)-1
Pigment epithelium-derived factor (PEDF)
Poly adenine dinucleotide phosphate-ribose polymerase (PARP)
Polymerase chain reaction (PCR)
Proliferative diabetic retinopathy (PDR)
Prostaglandins (PG)
Protein arginine methyltransferase 1 (PRMT1)
Protein kinase A (PKA)
Protein kinase C (PKC)
Reactive oxygen species (ROS)
Receptor for advanced glycation endproducts (RAGE)
Retinal capillary endothelial cell (RCEC)
Retinal endothelial cells (RECs)
Retinal nerve fiber layer (RNFL)
Silent Information Regulator (SIR)
Sirtuin (SIRT)
Streptozotocin (STZ)
Superoxide dismutase (SOD)
Transforming growth factor-beta (TGF-β)
Tumor necrosis factor-α (TNF-α)
Vascular endothelial growth factor (VEGF)

ABSTRACT

Diabetic retinopathy (DR) is one of the leading causes of blindness in the world. DR represents the most common microvascular complication of diabetes, and its incidence is constantly rising. The complex interactions between inflammation, oxidative stress, and the production of free oxygen radicals caused by prolonged exposure to hyperglycemia determine the development of DR. Sirtuins (SIRTs) are a recently discovered class of 7 histone deacetylases involved in cellular senescence, regulation of cell cycle, metabolic pathways, and DNA repair. SIRTs participate in the progress of several pathologies such as cancer, neurodegenerative and metabolic diseases. In DR, sirtuins 1,3,5 and 6 play an important role as they regulate the activation of the inflammatory response, insulin sensibility, and both glycolysis and gluconeogenesis. A wide spectrum of direct and indirect activators of SIRTs pathways (e.g. antagomiR, resveratrol, or glycyrrhizin) is currently being

developed to treat the inflammatory cascade occurring in DR. We focuse on the main metabolic and inflammatory pathways involving SIRTs and DR, as well as recent evidence on SIRTs activators that may be employed as novel therapeutic approaches to DR.

Keywords: AntagomiR, antioxidants, coumarin, diabetes mellitus, diabetic retinopathy, novel therapies, oxidative stress, resveratrol, sirtuin.

1. Introduction

In modern society diabetes mellitus (DM) is one of the most important health problems. There are two types of DM, type 1 in which pancreas does not produce enough insulin and type 2, the more common form, characterized by insulin-resistance. Insulin-resistance leads to the ineffective use of insulin, which causes the rise of blood glucose. Its incidence is growing at an alarming rate, and today about 422 million people worldwide have DM. There are 1.6 million deaths directly attributed to diabetes each year.[131] More than 45% of diabetic patients develop diabetic retinopathy (DR), which is the most common microvascular complication of both type 1 and type 2 diabetes. DR is one of the major causes of blindness in the world, and its incidence is progressively rising.[29] About 50% of patients develop DR after 10 years from the onset of the disease, and this goes up to 90% after 25 years.[16]

DR is a microangiopathy consisting of two phases: an early stage of non-proliferative diabetic retinopathy (NPDR) in which the vessel loss leads to ischemia, hypoxia, capillary leakage, and macular edema; and a later stage of proliferative DR (PDR), characterized by an increasing neovascularization mainly localized at the posterior pole.[131,29,16] Macular edema is the clinical manifestation of hyperpermeability of the retinal capillary network and breakdown of the inner blood-retinal barrier (BRB). This leads to extravasation of fluid and

other blood components into extracellular space.[81] The subsequent inflammation causes microruptures that contribute to the leakage of fluids into the retina.[81] The growth of new vessels distorts the microvasculature of the retina and eventually may cause retinal detachment.[3,16]

1. 1. Pathogenesis

DR is a multifactorial disease caused by numerous mechanisms. Inflammation, long-term hyperglycemia, augmented levels of advanced glycation end products (AGEs), and oxidative stress play major roles. All these factors are related and interact in the development of DR (Fig. 1).

Hyperglycemia causes high levels of AGEs by increasing non-enzymatic glycation. Thus, AGEs produce damage through modifications of soluble proteins, cellular matrix proteins, and DNA, altering cellular functions. AGE-modified proteins can elicit apoptosis through the binding of their receptors, called receptors for advanced glycation endproducts (RAGE).[97] This mechanism leads to the increase in production of reactive oxygen species (ROS) such as superoxide anion (O_2), hydrogen peroxide (H_2O_2) and nitric oxide (NO).[97,83] AGEs excess determines overactivation of hexosamine/polyol pathway with production of angiopoietin-2, activation of renin-angiotensin system and formation of new blood vessels.[40,135]

Moreover, activation of protein kinase C (PKC) results in stimulating neovascularization and endothelial proliferation, increasing vascular permeability, stimulating apoptosis, causing oxidative stress and abnormal blood flow.[32,109]

Nuclear factor-κB (NF-κB) is activated by oxidative stress caused by high glucose and AGEs. In fact, retinal pericytes showed NF-κB activation from high glucose levels, leading to a direct pro-apoptotic effect.[55,105]

Inflammation in DM patients has an important role in development of DR. Activation of NF-κB pathway promotes the expression of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-8 (IL-8), IL-6, and various pro-apoptotic regulators in retinal endothelial cells. TNF-α alters retinal endothelial barrier properties, reducing the expression and reshaping the distribution of the tight junction proteins claudin-5 and zonula occludens-1. This mechanism results in increased retinal vascular permeability.[7]

Inflammatory processes includes a major leukocytes adhesion and high level of cytokines and proinflammatory mediators. The consequent increase in vascular permeability and in production of ROS results in cellular damage and apoptosis.[3]

Also, augmented levels of IL-1β can be observed in retinas of diabetic animals from an increased activity of caspase-1 enzyme. IL-1β is one of the proinflammatory cytokines that activates NF-κB, inducing transcription of proinflammatory proteins, such as inducible nitric oxide synthase (iNOS), intercellular adhesion molecule-1 (ICAM-1) and cytokines.

Several studies demonstrated the upregulation of iNOS in retinas of diabetic murine models and patients.[141] Experimental mice, genetically deficient for iNOS did not develop diabetes-induced structural (including capillary degeneration) or functional (permeability) abnormalities of the retina.[60,140]

In the diabetic population, a high expression of cyclooxygenase-2 (COX-2) and prostaglandins (PG) is usually observed. The blockage of COX-2 causes the inhibition of vascular endothelial growth factor (VEGF), retinal vessel permeability, leukostasis, and death of retinal endothelial cells (RECs) cultured in diabetic-like concentrations of glucose.[120]

Leukocytes adhesion increases thanks to high level of expression of ICAM-1, P-selectin, and the leukocytes counter-receptor CD18 on the endothelium.[48] ICAM-1 overexpression responds to several stimulus such as VEGF, oxidative stress, polyadenine dinucleotide phosphate (ADP) ribose polymerase activation, and NF-κB.

Besides, α-4 integrin/CD-49 has been identified as an adhesion molecule whose inhibition reduces inflammation, activation of NF-κB, upregulation of VEGF and TNF-α, leukostasis and vascular leakage.[45]

Retina is particularly susceptible to oxidative stress because of its high content in polyunsaturated fatty acids, high oxygen demand, and glucose oxidation.[34]

Nicotinamide adenine dinucleotide phosphate oxidases (NOX), have an important role in DR. It increases the ROS formation determining the major oxidative stress associated with high expression of ICAM-1 and VEGF. NOX also increases leukocyte adhesion and breakdown of the BRB[104] and shows pro-inflammatory effects through the stimulation of endothelial cell proliferation.[108]

Hyperglycemia can activate the hexosamine/polyol pathway that is the result of hyperglycemia-induced oxidative stress. This causes a decrease of NADPH level and accumulation of ROS.[72]

High levels of glucose can inhibit glyceraldehyde 3-phosphate dehydrogenase producing high levels of glucosamine. The subsequent increase of H_2O_2 production results in heightened oxidation, changes in cell endothelium, increased vascular permeability, and angiogenesis.[132,15]

Oxidative stress represents a key factor in the development of DR that is characterized by an ischemic state that increases oxidative stress (Table 1). Consequently, the concentration of antioxidants as superoxide dismutase (SOD), glutathione, and lipid peroxide decreases, thus inducing oxidative damage to the retina.[12,15,132]

Today an increasing number of evidence shows neurodegeneration, glial activation and neuron apoptosis as key events in DR.

Significant pathogenetic mechanisms involved in retinal neurodegeneration in the diabetic eye are glutamate excitotoxicity, the downregulation of neuroprotective factors synthesised

by the retina, and the increase of oxidative stress and neuro-inflamation.[116] Transcriptional changes in activated microglia mediated by NF-kB and ERK signaling pathways result in release of various proinflammatory mediators, including cytokines, chemokines, caspases, and glutamate. These released factors cause increased apoptosis in retinal neurons resulting in thinning of the inner retina in diabetic mice.[4] The imbalance between neuroprotective factors--e.g. somatostatin, cortistatin, glucagon-like peptide 1, pigment epithelium-derived factor (PEDF)--is a major step in neural apoptosis and glial activation in diabetes.[4] PEDF is a peptide with neurotrophic properties and neuroprotective effects produced by retinal pigment epithelium cells. It determines the decrease of glutamate excitotoxicity by increasing the expression of glutamine synthetase, and by preventing glutamate transporter downregulation in retinal Müller cells.[41] Moreover, activation of microglia in the retina involves proliferation, migration, and changes in their morphology from ramified to amoeboid. This activation results in an inflammatory response that includes downregulation of cytokine IL-10 and growth factors and upregulation of various cytokines, chemokines, and neurotoxins.[4] Oxidative and inflammatory processes cause damages to neuron and glial cells by inducing alterations in the production and release of neurotrophic factors. To protect themselves, the suffering neurons, stimulate the production and release of VEGF by Müller cells. At the beginning VEGF acts as a neuroprotective factor. Subsequently, it determines vascular damages leading to new vessel proliferation.[106] Neurodegeneration processes are followed by changes in glial cells. Müller cells become less efficient in performing glutamate uptake or in converting glutamate to glutamine due to reduced levels of the enzyme glutamine synthetase.[41] Excess of glutamate stimulation causes an uncontrolled intracellular calcium response in postsynaptic neurons that results in cell death.[116] The characteristic change in glial cells in diabetic patients is called reactive gliosis. This process determines overexpression of glial acidic fibrillary protein (GFAP) by Müller cells, and impairment of

astrocyte functions, that migrate to the subretinal space. Subsequently, the activation of microglial cells and the release of cytokines contribute to neuronal cell death.[116] In Müller cells, under hyperglycemic conditions, glutamate metabolism is altered. This leads to an extracellular accumulation of glutamate in the neuroretina and neuronal apoptosis.[6]

2. Purpose of the review

At the end of this etiopathogenetic excursus, our goal is to summarize the current searches regarding the phenomena responsible for DR. Considering the data collected, we discuss in more detail the functions of ubiquitary enzymes called sirtuins (SIRTs) present within eukaryotic cells. In the last section, we have investigated several molecules that modulate the expression and activity of sirtuins as potential targets in the treatment of DR and DM.

3. Sirtuins overlook and general concepts

The SIRTs represent a group of 7 enzymes, SIRT1-7, with different distributions inside the eukaryotic cell, highly homologous to the silent information regulator (SIR) 2 found in yeast cells (*Saccharomyces cerevisiae*).[20,79] SIRTs have been associated with several cellular and metabolic processes. They are involved in the regulation of cellular plasticity and mechanisms of adaptation in various stress-related pathways.[119] SIRT1 and 2 both have nuclear and cytoplasmic location, SIRT3, 4 and 5 are mitochondrial proteins, SIRT6 and 7 are nuclear.[17] SIRT1 and 2 can also shuttle between cytosol and nucleus depending on the tissue development stage and cell cycle stage.[121,125]

SIRTs are included into class III histone deacetylases (HDACs), have a conserved central catalytic core, and differ in length of C- and N- terminal domains.[17] The catalytic core is characterized by a NAD+ binding region that intervenes in several different reactions.[22]

SIRTs most known activity is lysine deacetylation, but recent studies highlighted other activities such as succinyl, fatty acyl, glutaryl, and malonyl groups removal. SIRT 4 and 6 also show ADP-ribosyltransferase activity (Table 2).[17]

SIRT1,the most studied element of the SIRTs family, is highly localized in the nucleus of endothelial vascular cells, as well as the neurons and most embryonic tissues. SIRT1 combined with euchromatin plays a role in the maintenance of the chromatin stability.[96,118] SIRT1 is involved in the regulation of many patterns such as DNA repair, oxidative stress, angiogenesis, inflammation, and senescence. Recent studies have shown SIRT1 involvement in neurodegenerative, cardiovascular, tumorous, and metabolic diseases.[17,130,24] From a metabolic point of view, studies performed on murine models with induced overexpression of SIRT1 demonstrated a reduction in adiposity, insulin, and cholesterol serum levels. This led to the amelioration of insulin resistance, glucose tolerance, and obesity.[96] SIRT1 can be also induced by oxidative stress. In fact, its expression increases the levels of antioxidant enzymes determining, on a neuronal level, protection from axonal degeneration. On an hepatic level, SIRT1 is responsible for the protection from glucose intolerance and non- alcoholic fatty liver disease (NAFLD).[74,75]

SIRT1 deregulation has been implicated in the development of several ocular pathologies, such as cataract, age-related macular degeneration, DR, and glaucoma. SIRT1 deficient mice developed eye defects during the embryogenesis and hyperacetylation of p53 that led to a proapoptotic stimulus to the retinal cells.[143] Conversely, a recent study has demonstrated an increased expression of SIRT1 in the endothelium and pigmented epithelium of subjects affected by choroidal neovascularization membrane. This finding underlines a potential effect in the pathogenesis of vascular proliferative diseases.[77]

SIRT2 can be found in several tissues such as liver, prostate, kidneys and adipose tissue, but above all in the central nervous system. SIRT2 acts through the deacetylation of α-tubulin,

controlling the microtubules stability at a neuronal level. It has been suggested that SIRT2 may play a significant role in the development of neurodegenerative disorders.[71] Moreover, this enzyme controls the energetic state of the cell through the regulation of lipogenesis and insulin action. SIRT2 is considered a potential therapeutic target in liver steatosis, obesity, and DM.[33] Lastly, knockout mice for the SIRT2 gene have shown genome instability and the tendency to develop neoplasms.[110]

SIRT3 is the first SIRT discovered inside the mitochondria and participates in energy metabolism. Consequently, it's mostly expressed in high metabolism tissues: liver, brown adipose tissue, heart, brain, and muscles.[80] SIRT3 plays a control role in oxidative phosphorylation, fatty acid oxidation, amino acid catabolism and ketogenesis. Knockout mice for the SIRT3 gene did not show phenotypic alterations, but an altered insulin signaling pathway, and reduced glucose tolerance, as well as liver steatosis, dyslipidemia and obesity.[47,42] Moreover, the deacetylation activity of SIRT3 leads to the activation of SOD and the glutathione system preventing the accumulation of ROS inside the cell.[96]

The role of ***SIRT4*** is less known. Recent evidence suggests a potential role of this enzyme as a metabolism regulator. In fact, SIRT4 inhibits fat oxidation and, in pancreatic β-cells, reduces the insulin secretion in response to amino acids. It has been proposed that SIRT4 may act as a promoter in the development of NAFLD while SIRT3 and 5 may prevent it.[133] SIRT4 has a lysine-deacetylase function through which it controls leucine metabolism. A dysregulated leucine metabolism leads to high basal and stimulated insulin secretion that can cause the development of glucose intolerance and diabetes.[5] SIRT4 has a contradictory role in cancerogenesis as it may act both as a promoter and suppressor, depending on the tissue where the enzyme is expressed.[44]

SIRT5 is one of the most recent SIRT to be characterized. Even though it is considered to be a mitochondrial protein, it can also be found in the cytosol. This enzyme is highly expressed

in the liver, skeleton muscles, heart, kidney and brain. SIRT5 has a weak deacetylation activity and mainly performs its actions through desuccinylation, demalonylation and deglutaration.[103] Proteasome analysis showed that desuccinylation is responsible in heart and liver for amino acid metabolism, fatty acid oxidation, and tricarboxylic acid cycle.[98] Through the demalonylation activity, SIRT5 regulates gluconeogenesis and glycolysis in the liver.[95] The desuccinylation activity stimulates cellular resistance to oxidative stress through the activation of key enzyme such as SOD1, and the inhibition of cellular respiration.[63] Together with SIRT3, SIRT5 is responsible for the regulation of the urea cycle.[27] Lastly, the deregulation of SIRT5 may be responsible for the development of neurodegenerative diseases, as well as cancerous proliferation.[68,73]

SIRT6 is a nuclear enzyme responsible for chromatin modulation, DNA repair, and the preservation of genomic stability. For this reason, it has been linked to cancer and neurodegenerative diseases.[86] The role of SIRT6 in metabolic diseases is still poorly defined. Numerous recent evidence shows that SIRT6 expression is protective towards metabolic disorders, obesity and suppresses hepatic gluconeogenesis and glycolysis.[25] SIRT6 knockout mice tend to develop liver steatosis, inflammation, glucose intolerance, and insulin resistance. Similarly, expression of SIRT6 in mice allows the reduction of inflammatory responses in adipose tissue through the IL-4 mediated polarization of macrophages.[117]

On the other hand, in similar SIRT6 knockout murine models, it was demonstrated an increased glucose uptake in liver and skeletal muscles with a consistent reduction in TNF-α.[8] Zhong and coworkers demonstrated that SIRT6 can act as a suppressor of hypoxia induced factor-1α (HIF-1α) both with *in vitro* and *in vivo* models. They found that HIF-1α plays an important role as a direct transcriptional factor for many genes involved in the regulation of glucose fluxes. HIF-1α can improve glycolytic genes and glucose transporters, leading to a higher glucose cellular uptake. They observed that mice born with severe defects of SIRT6

were exposed to lethal hypoglycemia, while their liver showed a higher gluconeogenic activity to compensate for the hypoglycemia. The activation of HIF-1α, due to SIRT6 deficiency, led to a switch from aerobic to anaerobic metabolism. Likewise, SIRT6 determined the downregulation of HIF-1α at a transcriptional level. These results demonstrate how the expression of SIRT6 is key for the glucose metabolic balance inside and outside the cells.[142] ***SIRT7*** is an enzyme predominantly located inside the nucleolus that shows a weak deacetylase activity with specific substrates. SIRT7 is highly expressed in the liver, spleen, and testis.[53] SIRT7 knockout mice suffer from early embryonic lethality and shortened lifespan, as well as the accumulation of DNA damage.[126] Moreover, low expression of SIRT7 led to cardiac hypertrophy and hepatic steatosis.[112,124] SIRT7 can be potentially considered an oncogene due to its hyperexpression in numerous cancerous cell lines. SIRT7 expression appears to be vital for the maintenance of cancerous phenotype.[11]

It appears indisputable how SIRTs play a cardinal role in several different biological mechanisms and that they represent a potential target for future therapies. The next sections will specifically focus on the relationship between SIRTs and DR.

4. Sirtuins in diabetic retinopathy

4.1. SIRT1

The role of SIRT1 in DR has been extensively investigated, but the complex molecular interactions are not yet fully understood. Nevertheless, in recent years numerous studies have demonstrated a strong link between SIRT1 expression and the development of DR and PDR. MicroRNA (miRNA) are short RNA that negatively regulate the transcription of target mRNA by the degradation of target mRNA or by inhibiting the translational process. Recent

evidence have established a connection between several miRNA and the development of DM, DR, and other metabolic diseases.[10]

Mortuza and coworkers demonstrated, through a two-step experiment, that SIRT1 expression in DR is regulated at a post transcriptional level by miRNA-195. The first part focused on human retinal endothelial cells (HRECs) incubated with high concentration of glucose. Polymerase chain reaction (PCR) analysis showed an increased concentration of miRNA-195 and a lower concentration of SIRT1. Analogous results were obtained on retinas of diabetic murine models induced by a single injection of streptozotocin (STZ). Intravitreal injections of miRNA-195 antagomir normalized the retinal concentrations of both miRNA-195 and SIRT1. MiRNA-195 is responsible through the regulation of SIRT1 pathway of increase in fibronectin (FN) production, vascular permeability and oxidative stress, hallmarks of diabetic damage.[90] Similarly, hyperglycemia in RECs causes downregulation of SIRT1 followed by the reduction of mitochondrial antioxidant enzymes through forkhead box O1 (FOXO1) and p300 pathways.[89] Another regulator of the expression of SIRT1 in HRECs was found to be miRNA-23b-3p. The upregulation of this miRNA in DR leads to the reduction of SIRT1 transcription, determining the hyperacetylation of NF-κB. This transcription factor is responsible for the enhancement of inflammation that plays an important role in the development of diabetic microvascular complications. The replenishment of normal levels of SIRT1 interrupts the cellular metabolic memory induced by high glucose in retinal endothelial cells through the inhibition of NF-κB inflammatory pathways.[139]

Similar results can be reconducted to miRNA-211 that is overexpressed in serum of diabetic patients. MiRNA-211 is responsible for the development of RECs apoptosis, BRB breakdown, and increased vascular permeability. These actions are performed through SIRT1 inhibition, during the development of DR. Therefore, miRNA-211 could be used as biomarker for the early-onset of DR and as potential target therapy.[64]

MiRNA-34a is also involved in the development of DM via the activation of apoptosis and inflammation.[111,46] Researchers found that maternally expressed gene 3 (MEG3) is a long non-coding-RNA that promotes the expression of SIRT1. It has been demonstrated that MEG3 overexpression protects cellular lines from high glucose damages via the downregulation of miRNA-34a. This molecular mechanism determines the replenishment of SIRT1 expression and the inactivation of IL-1β, IL-6, TNF-α and NF-κB pathway.[122]

Likewise, miRNA-377 is responsible in HERCs exposed to high glucose for-cell migration, a key step in angiogenesis, as well as the release of proinflammatory molecules. MiRNA-377 is directly able to induce the NF-κB pathway and to inhibit SIRT1. The transfection of cellular lines with miRNA-377 antagomir allowed for the restoration of SIRT1.[21]

Lastly, a recent work demonstrated that miRNA-217 plays a role in the development of DR. Through the direct down regulation of SIRT1, miRNA-217 activates the inflammatory cascade in retinal pigment epithelial cells. The miRNA-217 inhibitor can reduce the expression of inflammatory factors such as IL-1β, IL-6, and TNF-α and determine the suppression of NF-κB in diabetic cellular models. These effects are mediated by the upregulation of SIRT1 expression.[134]

SIRT1, via the modulation of the acetylation status of p65, a subunit of NF-κB, downregulates the activation of metalloproteinase-9 (MMP-9). In DR, SIRT1 concentrations are consistently reduced. This leads to the activation of MMP-9 that initiates mitochondrial damage and oxidative stress, contributing to degenerative capillaries and pericyte loss.[56] In a similar study, based on *in vivo* and *in vitro* models of DR, the inhibition of SIRT1 expression determined the hyperacetylation of activation protein-1. This mechanism resulted into the hyperactivation of MMP-9 and the consequent development of microvessels damage and apoptosis of retinal capillary cells.[85]

Mishra and coworkers demonstrated that, in mice overexpressing SIRT1, the induction of diabetes by STZ injection was not accompanied by typical DR-related vascular and neuronal damages. Specifically, mice retained normal retinal thickness and electroretinogram responses. They showed normal vascular density and no signs of vascular leakage. Moreover, retinal cells appeared to be protected from hyperglycemia induced damage to the mitochondrial DNA and did not show hyperactivation of MMP-9.[84]

Oxidative stress increases the expression of protein arginine methyltransferase 1 (PRMT1) in retinal pigment epithelial cells of STZ-induced diabetic murine models. PRMT1 activation leads to a downregulation of SIRT1. This phenomenon is linked to the breakdown of the BRB, cellular apoptosis, and progression of DR.[52]

IL-17 is a proinflammatory cytokine secreted by lymphocytes T-helper 17. Its levels are systemically increased in diabetic patients, enhancing inflammatory response, angiogenesis, and consequent retinal damage. SIRT1 is known to modulate the expression of IL-17.[99] Liu *et al*. analyzed levels of SIRT1 and IL-17 in peripheral blood mononuclear cells (PBMCs) and in fibrovascular membranes samples. Increased concentrations of SIRT1 at a retinal level suggest a protective role of SIRT1 in PDR. In the PBMCs the authors detected high levels of IL-17 associated with reduced concentration of SIRT1.[70] Conversely, recent evidence demonstrated that the concentration of SIRT1 and SIRT3 and their mRNA are augmented in PBMCs of diabetic patients.[76]

High mobility group box 1 (HMGB1) is a transcription supporting protein mainly localized in the nucleus that is involved in inflammatory pathways, RECs apoptosis in RD, and the breakdown of BRB.[92] HMGB1 triggers the activation of RAGE and toll-like receptors that leads to the transcription of NF-κB. HMGB1 is upregulated in the vitreous and epiretinal membranes in patients with PDR.[88] SIRT1 inhibits the inflammatory response mediated by

HMGB1 activation. Moreover, resveratrol, a SIRT1 activator, reduces the expression of HMGB1 and exerts a protective role preventing vascular leakage and BRB breakdown.[87] Adding to the previous findings, protein kinase A (PKA) acts, through the activation of insulin growth factor binding protein-3, as an up regulator of SIRT1. In turn, SIRT1 upregulation leads to the reduction of HMGB1 in HRECs.[67]

Zeng and coworkers. identified the protective role of 3 glucagon-like peptide-1 (GLP-1) analogue, exendin-4 (EX-4), a regulator of blood glucose. EX-4 acts through the modulation of SIRT1 in rat retinal cells at the early stage of diabetes. In this murine model the authors highlighted elevated levels of ROS, responsible for retinal cells death, and decreased concentrations of SIRT1. The administration of EX-4 determined the reduction of H_2O_2-induced ROS and the replenishment of SIRT1 that led to a reduction of retinal cell death.[137]

SIRT1 has been also demonstrated to be a key regulator of cholesterol metabolism alongside with liver x receptor (LXR), mainly in the BRB. SIRT1 regulates the activation of LXR through deacetylation, which promotes insulin secretion and regulates body weight.[9] LXR is responsible for regulating cholesterol metabolism, suppressing NFκB pathways and containing immune response and inflammation. Interestingly, the administration of LXR ligands (i.e. GW3965) or SIRT1 activator (SRT1720) determined a reduction of inflammation molecular markers such as IL-6 and IL-1β.[39]

Endothelin-1 (ET-1), angiopoietin II, and transforming growth factor-beta (TGF-β) are vasoactive and growth proteins that play a role in the progression of several vascular diseases including diabetes. Specifically, ET-1 determines vasoconstriction and increases vascular permeability. TGF-β is a potent mitogen and controls cellular differentiation and apoptosis. Together they are responsible for the progression of fibrosis and the production of FN.[51,30]

Mortuza *et al*. demonstrated that SIRT1 regulates, through the modulation of p300, the

overexpression of ET-1 and TGF-β in HRECs exposed to high glucose concentrations and in STZ induced murine models.[91]

Overall, the role of SIRT1 in RD has been extensively studied, and even though a comprehensive understanding of its pathways is still lacking, SIRT1 will most likely play a cardinal role for the development of new therapies.

4.2. SIRT3

SIRT3 is primarily located inside the mitochondria and has been implicated in oxidative stress, metabolism and energetic homeostasis.[61]

Manganese superoxide dismutase (MnSOD) is one of the most important mitochondrial scavenging enzymes that regulates the levels of ROS. Previous studies demonstrated that MnSOD activity is reduced during the development of DM.[54] SIRT3 is able to implement a defense mechanism against hyperglycemia through the inactivation of the NF-κB signaling and the downregulation of gene encoding the apoptosis protein Bax. SIRT3 has also the ability to activate MnSOD in RECs by direct deacetylation following a reduction in the concentration of ROS.[31]

SIRT3 activates the anti-free radical pathway mediated by FOXO3. Similar to what was previously stated about SIRT1, Zeng and coworkers demonstrated a reduction in SIRT3 expression during early stages of DM in murine models. The combined increased expression of SIRT1 and SIRT3 exert a protective role toward the development of retinal damages. In addition, the administration of EX-4 determines an augmented expression of SIRT3, blocking ROS overproduction, thus protecting the retinal cells.[137]

Neovascularization participates as a cardinal step in the development of DR and PDR. It has been demonstrated that hyperglycemia in HRECs enhances the expression of VEGF, HIF-1α,

and insulin growth factor 1 as well as MMP-2 and MMP-9. All these molecules are considered to be markers of neovascularization.[114] After the exposure to high glucose concentrations, the induced hyperexpression of SIRT3 in HRECs resulted in decreased expression of neovascularization-related genes.[78]

SIRT3 and SIRT5 have proven to play a neuroprotective role in DR. In diabetic STZ-induced mice knockout for SIRT3 and SIRT5 genes, the exposure to hyperglycemia caused an accelerated neuroretinal dysfunction. The phenotype was not associated with the worsening of the typical signs of vascular disease. The authors hypothesize that a stage of neuronal dysfunction may precede the development of the vascular disease. Diabetic mice, knockout for either one between SIRT3 or SIRT5, did not show the same inner retinal dysfunctions. These findings suggest a redundant role of these SIRTs in retinal neuroprotection.[62]

4.3. SIRT6

Our current understanding of the mechanism regulating SIRT6 in RD is limited. SIRT6 is a nuclear SIRT binding chromatin that plays a role in inflammation, senescence, and oxidative stress.[49]

It has been demonstrated that oxidative stress determines the development of a senescence phenotype.[69] This phenotype can be summarized by a decrease of cellular growth and proliferation as well as the expression of senescence-associated β-galactosidase. Senescence is known to play a cardinal role in the development of vascular complications in DM such as DR.[14] Liu and coworkers highlighted that oxidative stress induced by H_2O_2 exposition in HRECs led to a reduced expression of SIRT6. Overexpression of SIRT6 determined a regression of the cellular senescence, while knockdown of SIRT6 simulated the effects of

H_2O_2. The authors suggested that the protective role of SIRT6 in RECs was mediated by the inactivation of retinoblastoma protein.[69]

It has been proposed that, previous to the development of the vascular disease, DR is characterized by a neurodegenerative step. The neurodegenerative process leads to the vascular disease through the breakdown of the BRB.[115] VEGF participates in the development of systemic vascular complications of diabetes and is a key trigger in PDR.[2]

Brain derived neurotrophic factor (BDNF) is a neurotrophin growth factor responsible for the maintenance of neuronal plasticity and neurogenesis. The reduction of BDNF at a retinal level is at least partially responsible for the neurodegenerative step of DR.[13]

Recent evidence demonstrated that, in early stages diabetic murine models, SIRT6 expression was suppressed, while retinal VEGF concentrations were increased. Adding to these findings, BDNF expression was suppressed with no evidence of vascular disease. This suggests that the deficiency in neuroprotective factors may be the starting point of the neurodegenerative step. This evidence is also strengthened by the observation of a significant reduction of the whole retinal thickness as well as some specific retinal layers in mice. VEGF concentrations were enhanced in Müller cells during exposition to high glucose in the presence of reduced level of SIRT6. This feedback that SIRT6 may have an epigenetic regulatory function on neurodegenerative signs characterizing early diabetes.[144] Additionally, SIRT6 knockout mice displayed glycolytic dysfunction related to an augmented rate of apoptosis in the inner retinal layers.[113]

5. Indirect action on SIRTs by specific agents for future therapeutic approaches in diabetes mellitus patients: AntagomiR, Fenofibrate, Extendin-4, MEG3, Kallistatin, Resveratrol, Flavonoids, Ginsenoside Rb1, Coumarins, Glycyrrhizin,

BGP-15, and Ergothioneine

5.1. AntagomiR vs. SIRT1

There is a strict association between miRNA and DR. SIRT1 could be the target gene of many miRNAs, e.g. miRNA-211, miRNA-195, miRNA-377, miRNA-34a, miRNA-125b, and miRNA-217. Hyperglycemia upregulates miRNAs that bind SIRT1 suppressing its expression. Some studies demonstrated that transfection with antagomiR can block this mechanism and ameliorate levels of SIRT1. This could be a future therapeutic approach. Thus, miRNA-211 and miRNA-195 are significantly up-egulated in diabetic retinal tissues and hyperglycemic human umbilical vein endothelial cells (HUVECs). They could suppress the expression of SIRT1 binding to the untranslated region (3'-UTR), leading to retinal vascular disorder and endothelial apoptosis associated with DR. With transfection of antagomiR-211 or antagomiR-195, this mechanism is blocked, and therefore they could alleviate diabetic retinal disorder and hyperglycemic HUVECs apoptosis by up-regulating the expression of SIRT1. [64,90]

5.2. Fenofibrate vs. SIRT1

Fenofibrate, an orally active peroxisome proliferator-activated receptor-α (PPAR-α) agonist, suppresses cellular metabolic memory of high glucose in diabetic retinopathy via a SIRT1-dependent signaling pathway. In two randomized trials conducted on patients with type 2 diabetes, fenofibrate slowed the progression rate of diabetic retinopathy and reduced the need of laser treatments of 36-38% in those patients with maculopathy and proliferative retinopathy. [18,50] In both trials the clinical benefit was related to effects including antiinflammatory, anti-angiogenic, and cell signaling. Fenofibrate is able to suppress the hyperglycemic-induced overexpression of NF-κB resulting in the inhibition of cellular

apoptosis in HRECs. The inhibitory effect of fenofibrate on high glucose-induced NF-κB is SIRT1-dependent. It upregulates SIRT1 expression through the activation of PPAR-α in HRECs. Moreover, fenofibrate can revert the cellular metabolic memory induced by hyperglycemia, a key step in the development of inflammation. [138]

5.3. GLP-1 analogue or EX-4 vs. SIRT1 and SIRT3

Zeng and coworkers demonstrated in a streptozotocin-induced diabetic murine model the effects of EX-4. Intravitreal injection of GLP-1 analogue, EX-4, can suppress oxidative stress, reduce gliosis, decrease apoptotic factors, maintain the integrity of the BRB, reduce NOX expression and increase clearance of superoxide. EX-4 increases SIRT1 and SIRT3 expression, reducing both cellular death and ROS level in the retinas. EX-4 increases the NAD+/NADH ratio and upregulates nicotinamide phosphoribosyltransferase and thus upregulates SIRT1. The treatment with EX-4 increases SIRT1 and SIRT3 expression, whereas hyperglycemia decreases them, resulting in retinal cells protection from ROS. Additionally, EX-4 intravitreal injections ameliorated the rat visual functions measured by electroretinogram. [137]

5.4. MEG3 vs. SIRT1

MEG3 is a long non-coding-RNA (Lnc-RNA) gene that is able to bind miRNA-34a, regulating the expression of the gene SIRT1. High glucose inhibits MEG3 and enhances the levels of miRNA-34a decreasing level of SIRT1. Overexpression of MEG3 decreases miRNA-34a levels and promotes SIRT1 expression. This leads to the inhibition of cellular apoptosis and secretion of inflammatory cytokines (IL-1β, IL-6, and TNF-α). Moreover, high glucose activates NF-κB signaling pathway and the overexpression of MEG3 blocks it by

inhibiting the expression of p-65 in human retinal pigment epithelial cells (ARPE-19). Overexpression of MEG3 and knockdown of miRNA-34a results in increased Bcl-2/Bax ratio and decreased apoptosis rates. MEG3 inhibits both inflammation and apoptosis through the regulation of NF-κB pathways and Bax/Bcl-2 ratio by targeting miRNA-34a/SIRT1 axis in ARPE-19.[122] In early stages of diabetes Müller cells develop a reactive gliosis, leading to the production of glial acidic fibrillary protein,[59] IL-6, IL-1β, TNF-α and VEGF.[57] Tu and coworkers focused their research on the proinflammatory role of Müller cells in DR. They demonstrated a potential role of melatonin in the reduction of inflammation and in protecting Muller cells from hyperglycemia injury through the enhancement of antioxidant defences. They conducted their experiment on a diabetic murine model and human Müller cultured cells incubated with high glucose concentrations. The treatment with melatonin showed the upregulation of MEG3/miR-204/Sirt1 axis. This led to the inhibition of proinflammatory cytokines production in Müller cultured cells. In the retinas of diabetic mice melatonin led to an alleviation of BRB damage.[123]

5.5. Kallistatin vs. SIRT1

Kallistatin a tissue kallikrein-binding protein and a serine proteinase inhibitor, contains two sites: a heparin-binding site and an active site. The active site is crucial for inhibiting tissue kallikrein activity, and stimulating endothelial nitric oxide synthase (eNOS), SIRT1 expression and catalase synthesis. Kallistatin blocks miRNA-34a synthesis preventing miRNA-34a mediated inhibition of SIRT1 and eNOS expression in both endothelial progenitor cells and STZ-induced diabetic mice. Kallistatin stimulates antioxidant gene expression such as SIRT1, eNOS, catalase and SOD2. Kallistatin inhibits endothelial senescence and oxidative stress through SIRT1-mediated eNOS pathway. In fact, an inhibitor

of SIRT1 activity blocks kallistatin-stimulated eNOS expression, whereas a NOS inhibitor has no effect on kallistatin-stimulated SIRT1 expression.[37] Guo and coworkers demonstrated that kallistatin upregulates lethal-7 gene (Let-7g), preventing miRNA-34a-mediated inhibition of SIRT1-eNOS pathway in HUVECs cultures. The results show that kallistatin reduces endothelial senescence, oxidative stress, and inflammation.[36]

5.6. Resveratrol and AICAR vs. SIRT1

Resveratrol (3,5,4-trihydroxystilbene) is one of the polyphenolic phytoalexins found in red wine and grape skin. It has various effects such as antioxidant, antitumorigenic, antiangiogenic, and neuroprotective. It suppresses ocular inflammation in endotoxin-induced uveitis through its antioxidative property and SIRT1-activating action, both of which lead to inhibition of the NF-κB pathway. It prevents neuronal damage in diabetic retina and has a neuroprotective role on light-induced retinal degeneration. Resveratrol also increases the activity of adenosine monophosphate (AMP)-activated protein kinase (AMPK). Even if the relationship between SIRT1 and AMPK in the pathogenesis of DR remains to be elucidated, systemic administration of the AMPK activator resveratrol or 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) increases SIRT1 activity. Moreover, in STZ-induced diabetic mice models AMPK activation leads to significant suppression of NF-κB, which regulates gene expression of various proinflammatory molecules responsible for the pathogenesis of DR. Therefore, AMPK activator resveratrol could be used as a potential therapeutic agent for DR.[58] Some compounds similar to resveratrol such as SRT1460, SRT1720 and SRT2183 show similar effects as they bind to a SIRT1–peptide substrate complex and enhance its catalytic activity. These compounds improve insulin sensitivity, lower plasma glucose, and increase mitochondrial capacity in mice models. SIRT1 activators

improve whole-body glucose homeostasis and insulin sensitivity in adipose tissue, skeletal muscle and liver. The SIRT1 activators increases cell survival by stimulating SIRT1-dependent deacetylation of p53.[82]

5.7. Isoflavones vs. SIRT1

Isoflavones are a type of naturally occurring isoflavonoids, many of which act like phytoestrogens in mammals. Isoflavones are produced almost exclusively by the members of the bean family, *Fabaceae* o *Leguminosae*. They can promote the activation and/or expression of SIRT1, an activator of peroxisome proliferator-activated receptor gamma coactivator (PGC)-1 that protects against oxidative stress, increases mitochondrial number, and intracellular ATP. [100] PGC-1 has shown the ability to regulate mitochondrial biogenesis, implement the number of mitochondria and ameliorate cellular respiration. These considerations suggest that PGC-1 could be a target against oxidative stress insult.[101] SIRT1 can increase PGC-1 expression and activity. [93] Isoflavones such as daidzei or ganistein shown a protective role against metabolic disease in mice models.[1] Rasbach and coworkers conducted an experiment on culture of renal proximal tubules cells (RPTC) exposed to different isoflavones for 48 h. They demonstrated the up-regulation of PGC-1 that promotes the biogenesis of mitochondria and the increase of cellular respiration and ATP levels. Isoflavones derivatives also increased SIRT-1 activity.[100] Further studies are needed to evaluate if isoflavonoids could be a potential future therapeutic strategy in DR.

5.8. Ginsenoside Rb1 vs. SIRT1 and SIRT3

Ginsenosides or panaxosides are a class of natural product steroid glycosides and triterpene saponins. Compounds in this family are found almost exclusively in the plant genus *Panax*

(ginseng). Ginsenoside Rb1 has many properties such as antioxidative, anti-inflammatory, antidiabetic, cardio- and neuroprotective. Whether Rb1 is retinoprotective is unclear. Fan and coworkers conducted an experiment on rat retinal capillary endothelial cells (RCECs) cultured in normal, high glucose, and high glucose plus Rb1. Rb1 increased survival rate of cells cultured in high glucose conditions. Rb1 treatment determined a reduction of ROS production, ameliorating high glucose-induced endothelial damage and reducing high glucose-induced oxidative stress. The effects of Rb1 appear to be mediated by the activation of nicotinamide mononucleotide adenylyltransferase 1 (NMNAT1)-nicotinamide adenine dinucleotide (NAD+)-poly ADP-ribose polymerase (PARP)-SIRT axis. Specifically, ginsenoside Rb1 is able to inhibit the PARP mediated cell death that triggers under hyperglycemic conditions, restoring NAD+ levels. Finally, it maintains the intracellular redox balance by increasing the NADPH and glutathione levels. Further studies are warranted to demonstrate whether Rb1 may be able to exert similar effects on human endothelial cells.[28]

5.9. Coumarins vs. SIRT1

Dao and coworkers isolated four new terpenylated coumarins from a methanol extract of the stem bark (compound 1-4) of *Ailanthus altissima*, a deciduous tree belonging to the family *Simaroubaceae*, native of both northeast and central China, as well as Taiwan. The *in vitro* study was performed on human embryonic kidney cells. These compounds determined an increase in SIRT1 deacetylation activity that led to a decrease in p53 transcriptional activity. Coumarins also increased the NAD-to-NADH ratio by more than 50%. The authors therefore suggest the 4 compounds as potent natural activators of SIRT1. It is still to be determined whether these results may be translated to *in vivo* experiments and if coumarin derivatives may be employed as a natural treatment for metabolic diseases such as diabetes.[23]

5.10. Glycyrrhizin vs. SIRT1

Glycyrrhizin is the chief sweet-tasting constituent of *Glycyrrhiza glabra* (liquorice) root. Liu and coworkers demonstrated in diabetic rat models the beneficial effect of glycyrrhizin in DR. It reduces the diabetes-induced permeability and maintains normal retinal thickness and cell count . Glycyrrhizin inhibits HMGB1 through direct binding, protecting the retinal vasculature. It reduces ROS, TNF-α and IL-1β.[66] The same authors demonstrated that glycyrrhizin significantly increases SIRT1 expression in diabetic rats. The increased SIRT1 expression led to the deacetylation of HMGB1 and the reduction of its cytoplasmic and extracellular inflammatory effect on retinal vasculature. [65]

5.11. BGP-15 vs. SIRT1

N-[2-hydroxy-3-(1-piperidinyl) propoxy]-3-pyridinecarboximidamide, dihydrochloride (BGP-15) is a PARP inhibitor and insulin sensitizer that decreases levels of ROS, lipid peroxidation and damage to DNA. Wachal and coworkers demonstrated that BGP-15 is able to counteract the retinal function-deteriorating effect of diabetes by elevating SIRT1 expression and decreasing MMP-9 expression in diabetic rat eyes. BGP-15 effects were comparable to the more known antidiabetic drugs such as pioglitazone and metformin. Interestingly, BGP-15 was able to counteract the deteriorating effects of hyperglycemia, measured with electroretinography, in a similar manner as the antidiabetic drugs. These results show that hydroximic acid derivative may be employed as a new drug to counteract DR development.[129]

5.12. Ergothioneine vs. SIRT1 and SIRT6

Ergothioneine (EGT) is a compound containing a 2-thioimidazole moiety synthesised only by some fungi and bacteria. It acts as a dietary antioxidant that has shown a protective action against cardiovascular, neurodegenerative and metabolic disease.[38] D'Onofrio and coworkers demonstrated beneficial effects exerted through the upregulation of SIRT1 and SIRT6 expression and the downregulation of p66Shc and NF-κB against hyperglycemia-induced senescence in cows' pulmonary artery endothelial cells. EGT reduces high glucose-mediated cytotoxic effect in a dose-dependent manner. Moreover, EGT has also SIRT-dependent beneficial effects. SIRT1 protects blood vessels from hyperglycemia-induced endothelial dysfunction by downregulating p66Shc, that stimulates ROS generation and oxygen reduction with H_2O_2 formation. EGT can both upregulate SIRT1 and downregulate p66Shc. In high glucose levels SIRT6 deficiency increases the activity of NF-κB and the expression of proinflammatory cytokines. EGT reduces both high glucose-mediated downregulation of SIRT6 and upregulation of NF-κB. The effects of EGT were SIRT-mediated. In fact, the use of an inhibitor of SIRT1 or the silencing of SIRT6 determines the loss of efficacy of EGT.[26]

6. Discussion

We have highlighted the characteristics of the SIRTs, enzymes ubiquitously distributed from eubacteria to mammals that have remarkable properties in preventing diseases and aging.[17,20,79,107,121,125] They regulate several cellular biochemical factors including inflammation processes, DNA repair, fat differentiation, carcinogenesis, glucose output, insulin sensitivity, fatty acid oxidation, neurogenesis, and aging.[24,74,75,96,118,119,130] Recent studies, indicated that their main biochemical activities are deacetylation, demalonylation, desuccinylation, O-ADP-ribosylation, and deproprionylation.[17,22] The capacity to modify the activity of these enzymes

may open new therapeutic roads to improve pathological conditions involving the SIRTs. Nowadays, several SIRTs activators have been identified and could be employed as novel therapeutic approaches in DM.

SIRT1 is the best-known protein and can interact with numerous substances. For example, the most promising therapeutic approaches include molecules capable of acting on miRNA. In fact, antagomiR can block or significantly reduce the expression of different miRNAs.[10,21,64,90,122,134,139] After the activation phase, the metabolic cascade follows through complex mechanisms of inflammation and oxidative stress that can lead to proliferating vitreoretinopathy associated to retinal detachment and finally to the loss of visual capacity. AntagomiR can act upstream of the metabolic cascade and slow down or block the pathological process.

Similarly, MEG3, an Lnc-RNA gene, can bind miRNA-34a regulating the expression of the gene SIRT1.[122] Moreover, the treatment with EX-4 increases SIRT1 and SIRT3 resulting in retinal cells protection from ROS.[137] Resveratrol and similar compounds such as SRT1460, SRT1720 and SRT2183 bind to a SIRT1–peptide substrate complex and enhance catalytic activity, insulin sensitivity, mitochondrial capacity, and cell survival by stimulating SIRT1-dependent deacetylation of p53.[58,82,43] Resveratrol has a neuroprotective role, as it increases the activity of AMPK. This leads to a significant suppression of NF-κB, which regulates gene expression of various proinflammatory molecules responsible for the pathogenesis of DR.[75]

The concept of DR as a microvascular disease alone has evolved. Neurodegeneration also plays an important role as an early event in the pathogenesis of diabetic retinopathy. Glial activation and neuron apoptosis are the two prominent hallmarks. Microglial cells are activated by a complex interplay between different cell types and numerous pathological pathways. The exact pattern of microglial activation in DR is still unknown. Müller cells play important effects in the glutamate metabolism, extracellular ionic balance, and neuronal

function. Firstly, the activity of glutamine synthetase in Müller cells is reduced. Secondly, glutamate oxidation to alpha-ketoglutarate is impaired. Finally, glutamate uptake by Müller cells is diminished. Low-grade inflammation, immune cell activation, extracellular glutamate accumulation and an imbalance of local production of neurotrophic factors are crucial in the development of retinal neurodegeneration. Glial activation and neuron apoptosis are the two prominent hallmarks of this condition and these changes have been observed to occur before overt microangiopathy.[116,136]

Today the measurement of retinal nerve fiber layer (RNFL) and ganglion cell layer (GCL) thickness with optical coherence tomography (OCT) could serve as a simple early sign of neurodegeneration in diabetic retina.[6]

Verma and coworkers correlated the OCT measured foveal thickness and the photoreceptor layer at the foveal centre, with the microperimetric mean retinal sensitivity of central 20 degrees. They report that the thickness of the foveal area and photoreceptor layer reduces simultaneously with the retinal sensitivity.[128]

Further studies demonstrated that the macular inner retinal layer RNFL, GCL, and internal plexiform layer (IPL) were thinner in patients with minimal DR compared to controls, in an unselected population of patients with type 2 diabetes.[19]

Moreover, in subjects with diabetes, retinal ganglion cells are vulnerable to damage prior to the onset of apparent microvascular DR lesions. The retinal GC damage is progressive, with subsequent severe forms of DR. OCT measures indicative of GC-IPL and RNFL thinning, but not outer retinal thinning, were associated with diabetic subjects with no clinically apparent DR. In summary, retinal GC loss is present in subjects with diabetes and no DR and is progressive in moderate or severe DR.[94,35]

GCL and RNFL thickness was reduced in patients with diabetes without diabetic retinopathy, suggesting that neuroretinal changes precede vascular signs of diabetic retinopathy.[127]

Most SIRTs exert numerous actions in neuronal cells. The scientific literature is full of articles on neurodegenerative diseases, even severe ones, associated with microvascular damage. The activators of the SIRTs, as potential therapeutic targets for treating DR, could be used to slow down or stop the degenerative process of the disease. We know that a compound can regulate, under certain experimental conditions, the expression or activity of SIRTs, but this does not automatically make it a potential therapeutic strategy. This may be case for Glycyrrhizin and BGP-15, as argued by from us previousl, but not for coumarins and isoflavones.

Liu and coworkers demonstrated in diabetic rat models the beneficial effect of glycyrrhizin in DR. It reduces ROS, TNF-α, IL-1β, cleaved caspase 3, retinal vasculature permeability, and maintains normal retinal thickness and cell count.[66] Glycyrrhizin increases SIRT1 expression in diabetic rats and inhibits HMGB1 through the inhibition of inflammatory pathways activated by HMBG1 .[65,66]

Furthermore, Wachal and coworkers demonstrated that BGP-15 is able to counteract the retinal function-deteriorating effect of diabetes by elevating SIRT1 expression and decreasing MMP-9 expression in diabetic rat eyes. BGP-15 effects were comparable to the most known antidiabetic drugs. BGP-15 was involved in the different mechanisms of action increasing SIRT1 and retinal function and was effective in decreasing blood glucose and MMP9. The beneficial effects were related to the increase of the mitochondria-defender SIRT1 expression and, at the same time, to the decrease of the mitochondria-destroyer MMP9 expression in the diabetic eye.[129]

In this regard, targeted studies will be needed to broaden our knowledge, and promote the therapeutic applications of products capable of implementing the beneficial effect of SIRTs in diabetic patients.

7. Conclusions

In accordance with the above reports, an increased number of studies have confirmed the actions of SIRTs in numerous biomolecular processes related to oxidative stress.

The use of various protective molecules able to modulate the beneficial effect of SIRTs could be helpful in the cellular stress response. To the best of our knowledge, current initial studies have been carried out only in laboratory on animal models and cultured human cells.

Therefore, human clinical trials need to be implemented in order to see if there is improvement in the clinical course of patients with DR.

8. Method of literature search

A literature search was performed to collect relative information regarding the role of the pathophysiology of DR using PubMed/Medline. Publications from 2000 to 2020 were considered for this study. The key words used for the search were: *"diabetes mellitus, diabetic retinopathy, hyperglycemia, oxidative stress, cytokines, retinal endothelial cells apoptosis, antioxidants, free radical damage, sirtuin, novel therapies, antagomiR, coumarin, resveratrol".* All relevant articles were included. Reference lists from the selected articles were checked to obtain further informations. Articles written in English were considered. Types of publications considered for our manuscript were mainly research and reviews.

9. Disclosure

The authors reported no proprietary or commercial interest in any product mentioned or concept discussed in this article.

Author Contributions

Conceptualization, MN and AL; writing original draft preparation, MA, LA and GT; writing review and editing, MN, MA, and LA; data curation, MS, and AG; supervision AL.

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Figure Legend

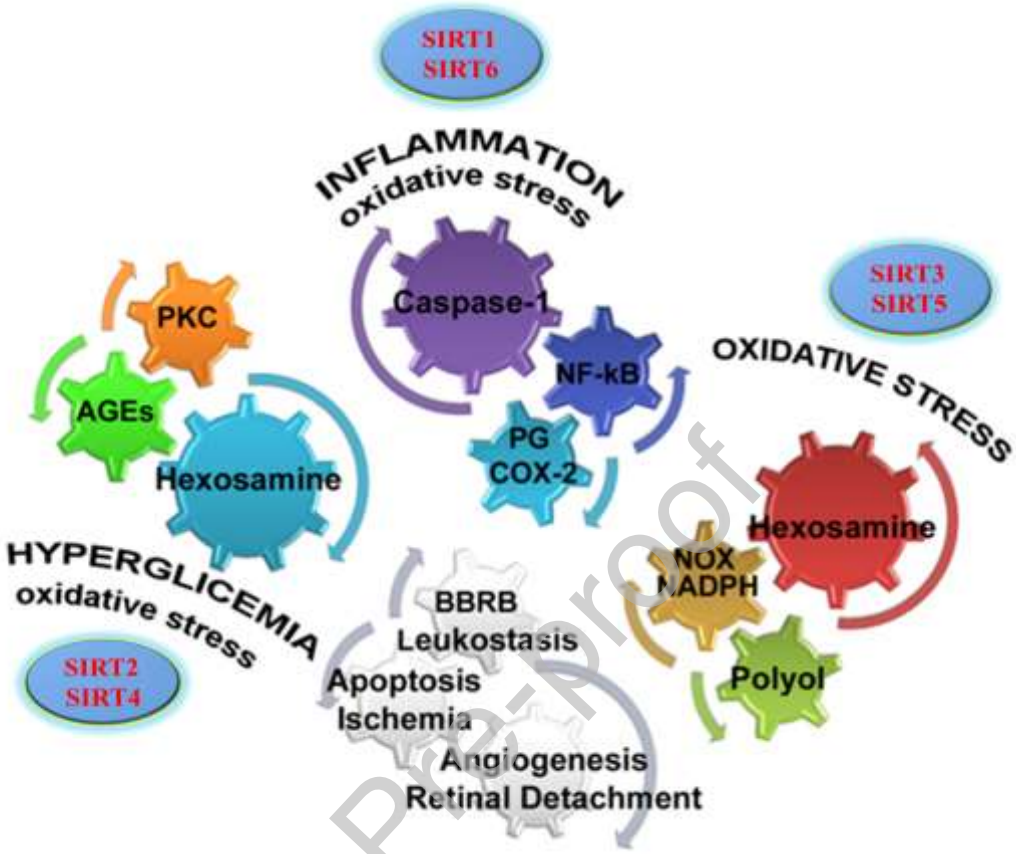

Figure 1 - Diabetic retinopathy (DR) is a multifactorial disease. Inflammation, long-term hyperglycemia, and oxidative stress play a main role. All these factors are strictly related and corroborate each other in the development of DR. Sirtuins (SIRT1-6) involved in the regulation of cellular plasticity and mechanisms of adaptation in various stress-related pathways. Protein kinase C (PKC); advanced glycation end products (AGEs); nuclear factor-κB (NF-κB); prostaglandins (PG); cyclooxygenase-2 (COX-2); nicotinamide adenine dinucleotide phosphate (NADPH); nicotinamide adenine dinucleotide phosphate oxidase (NOX); breakdown of the inner blood-retinal barrier (BBRB).

Table 1 - Etiopathogenetic pathways in diabetic retinopathy (DR) activated by hyperglycemia, inflammation, and oxidative stress.

DIABETES MELLITUS-PHASES	HYPERGLICEMIA, INFLAMMATION, AND OXIDATIVE STRESS
Dysregulation mechanisms	**Increased hexosamine/polyol pathway → decrease in NADPH/SOD→ increased NoxNADPH oxidase → overproduction of ROS (O_2 / H_2O_2 / NO) → → Molecular changes → progression of Diabetic Retinopathy (DR)**
Systems and molecules involved	Increased AGEs and RAGE, PPAR-α, activation of PKC, caspase-1 enzyme, renin-angiotensin system, diacylglycerol, angiopoietin-2, endothelin-1, lypoxygenase, iNOS, HMGB1, ICAM-1, PG, NO, COX-2; VEGF, IL-1β, IL-8, IL-6, IL-17, MMP-9, HIF-1, TNF-α, IGF1, NF-κB, PEDF, P-selectin.
BBRB and non proliferative DR	Neuronal dysfunction, leukostasis, vasoconstriction, hypoxia, retinal ischemia, aneurysms and telangiectasias, leakage and edema, lipid deposition, retinal hemorrhages, thrombosis.
Progression of DR	RECs death and vascular endothelial dysfunction, growth dysregulation.
Proliferative DR	Apoptosis of retinal cells, fibrosis, fibrinolysis, angiogenesis, vitreoretinal proliferations.
Final stage worsening	Macular edema, vitreal alterations, vitreous hemorrhages, vitreomacular traction, retinal detachment.

NADPH: nicotinamide adenine dinucleotide phosphate; SOD: superoxide dismutase; ROS: reactive oxygen species; AGEs: advanced glycation end products; RAGE: receptor for advanced glycation end products; PPAR-α: peroxisome proliferator-activated receptor-α; PKC: protein kinase C; iNOS: inducible nitric oxide synthase; HMGB1: high mobility group box 1; ICAM-1: intercellular adhesion-1; PG: prostaglandins; NO: nitric oxide; COX-2: cyclooxygenase-2; VEGF: vascular endothelial growth factor; IL: interleukin; MMP-9: metalloproteinase-9; HIF-1: hypoxia-inducible factor-1; TNF-α: tumor necrosis factor; IGF1: insulin-like growth factor; NF-κB: nuclear factor-kappa B; PEDF: pigment epithelium-derived factor; BBRB: breakdown of the blood-retinal barrier; RECs: retinal endothelial cells.

Table 2 - Sirtuins (SIRTs) are included into class III histone deacetylases (HDACs), play a cardinal role in several biological mechanism, and in large part increase the antioxidant enzymes. Main characteristic of mammalian SIRTs.

SIRTs	Location and principal role	Highly localized	Patterns of regulation	Involvement in diseases	Main metabolic actions
SIRT1	Nuclear and cytosolic. Chromatin stability.	Endothelial vascular cells, neurons, tissues of embryos	DNA repair, reduces angiogenesis and inflammation, regulates senescence	Neurodegenerative, cardiovascular, hepatic tumorous, metabolic	Reduction in adiposity, insulin, and cholesterol serum level
SIRT2	Nuclear and cytosolic. Neuronal microtubules stability.	CNS, prostate, liver, kidneys, adipose tissue	Controls the energetic state by means lipogenesis and insulin	Neurodegenerative, DM, tumorous, steatosis, obesity, liver	Lipogenesis and insulin action
SIRT3	Mitochondrial. Energy metabolism.	Liver, brown adipose tissue, heart, brain, muscles	Oxidative phosphorylation, fatty acid oxidation, amino acid catabolism, ketogenesis	Liver steatosis, obesity	Glucose tolerance, insulin signalling pathway. Regulates the urea cycle
SIRT4	Mitochondrial. Metabolism regulator.	Pancreatic β-cells	Inhibits fat oxidation, reduces the insulin secretion in response to amino acids	Glucose intolerance and DM, promotes or suppresses carcinogenesis	Lysine-deacetylase function, through which it controls leucine metabolism in the stimulated insulin secretion
SIRT5	Mitochondrial and cytosolic. Desuccinylation, demalonylation, deglutaration, deacetylation.	Liver, skeleton muscles, heart, kidney, brain	Desuccinylation is responsible, in heart and liver, for amino acid metabolism, fatty acid oxidation and tricarboxylic acid cycle	Neurodegenerative, cancerous proliferation	Regulates gluconeogenesis, glycolysis, and cycle of the urea, actives enzyme of the resistance to oxidative stress
SIRT6	Nuclear. Chromatin stability.	Neurons, adipose tissue, liver	DNA repair, reduction of inflammatory responses in adipose tissue through the IL-4 mediated polarization of macrophages	Neurodegenerative, tumorous, obesity, protective towards metabolic disorders	Suppresses glycolysis, gluconeogenesis, protects from glucose intolerance and insulin resistance
SIRT7	Nucleolus. Weak deacetylase activity.	Liver, spleen, testis	Low expression led to cardiac hypertrophy and hepatic steatosis.	Hyperexpression in cancerous cell lines.	Promotes adipogenesis by inhibiting Sirt1

AMD: age-associated macular degeneration; DR: diabetic retinopathy; CNS: central nervous system; DM: diabetes mellitus.