We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 4

Oxidative Stress Biomarkers for Diabetic Retinopathy and Medical Management Affecting Oxidative Stress

Ines Cilenšek, Sara Mankoč Ramuš,
Mojca Globočnik Petrovič and Daniel Petrovič

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/63353

Abstract

Changes in dietary habits and lifestyles associated with rapid economic growth have dramatically increased the incidence of diabetes and related vascular complications. Diabetic retinopathy (DR), a microvascular complication of diabetes, is associated with both environmental and genetic factors. Several metabolic abnormalities are implicated in its pathogenesis; however, the exact mechanism remains to be determined. Among them, oxidative stress is expected to play an important role.

Environmental, genetic, and epigenetic factors affecting the oxidative stress responsible for DR are reviewed in this paper. The knowledge about genetic biomarkers of DR is quite extensive, whereas the awareness about epigenetics and epigenetic markers is only beginning to be understood.

Modulation of epigenetic changes by pharmaceutical means may provide a potential strategy to retard the progression of DR. In addition to the intense medical management, these strategies include dietary measures (antioxidants) and the introduction of epigenetic drugs, such as inhibitors of DNA methylation and histone demethylases.

Keywords: oxidative stress, diabetic retinopathy, gene polymorphisms, epigenetics, medical management

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. The prevalence of diabetes has

INTECH
open science | open minds

© 2016 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

reached global epidemic proportions. According to the Internal Diabetes Federal (IDF) data, there were 382 million people living with diabetes in 2013, whereas a further 316 million with impaired glucose tolerance are at high risk of the disease—an alarming number that is set to reach 471 million by 2035. Type 2 diabetes (T2DM) is the most prevalent type of diabetes. It is by far the most common form of diabetes in elderly people, but is increasingly seen in children and adolescents IDF, 2013 as well. The causes of the T2DM epidemic are embedded in a very complex group of genetic and epigenetic systems interacting within an equally complex societal framework that determines behavior and environmental influences [2]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1].

2. Pathogenesis of DR

Chronic elevation in circulating blood glucose damages blood vessels, which results in many micro- and macrovascular complications. DR is one of the major microvascular complications affecting the vision and is the leading cause of blindness in working-age adults [3]. It progresses from mild nonproliferative abnormalities, characterized by increased vascular permeability, to nonproliferative diabetic retinopathy (NPDR), characterized by vascular closure, to proliferative diabetic retinopathy (PDR), characterized by the growth of new blood vessels in the retina and the posterior surface of the vitreous [4] (**Figure 1**).

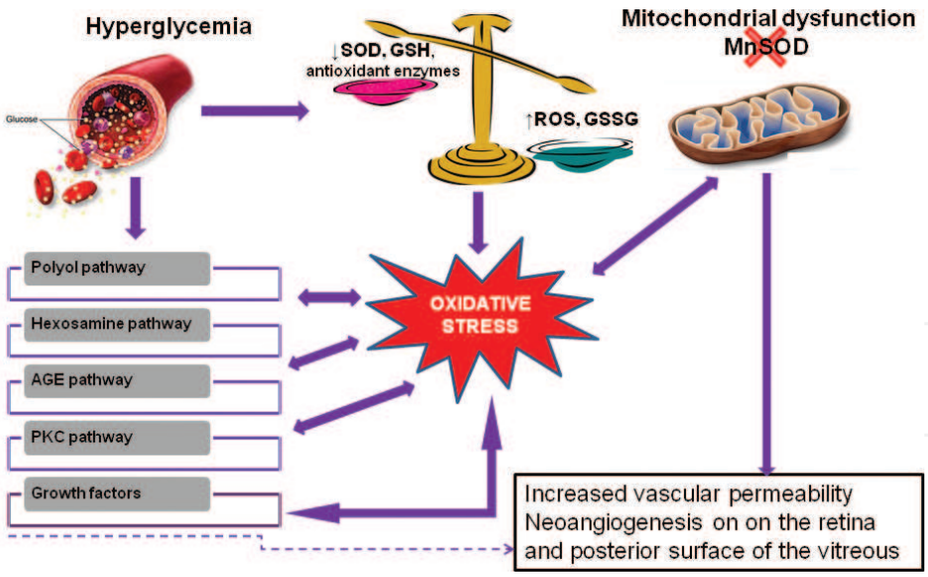

Figure 1. Major pathways implicated in the development of diabetic retinopathy.

It is a multifactorial condition for which the pathophysiology is incompletely understood [5]. There are many pathophysiological mechanisms through which diabetes might affect the initiation and promotion of the many underlying pathologies associated with DR [6]. The strong impact of hyperglycemia on DR incidence was confirmed by the Diabetes Control and

http://dx.doi.org/10.5772/63353

Complications Trial (DCCT) and the UK Prospective Diabetes Study (UKPDS) clinical trials [7,8]. Hyperglycemia activates several well-characterized biochemical pathways that play a significant role in the development of DR [9]. Major pathways implicated in the development of DR are the polyol pathway, protein kinase C (PKC) activation, accumulation of advanced glycation end products (AGEs), oxidative stress, activation of the hexosamine biosynthesis pathway, and growth factors (**Figure 1**) [3,6,9–11]. The activation of these pathways, in turn, leads to the secondary production of reactive oxygen species (ROS) and the consequent increase in oxidative stress that affects carbohydrates, lipids, proteins, and nucleic acids [9]. The oxidative stress plays a pivotal role in cellular injury from hyperglycemia.

3. Biomarkers

A biomarker is a measurable indicator of a specific biological state, usually one relevant to the risk, presence, severity, prognosis, or predicted therapeutic response of the disease. In medicine, biomarkers are often compounds isolated from serum, urine, or other fluids that can be used as an indicator of the presence or severity of a particular disease state. Molecular biomarkers can themselves take many forms, and as a consequence there are many strategies available for their discovery and validation. Transcriptional profiling, DNA methylation studies, and kinase sequencing have shown a strong potential for biomarker discovery in several disorders; metabolomics approaches are beginning to show promise for metabolic diseases, such as DR. Molecular biomarkers (DNA gene polymorphisms, RNA gene polymorphisms, proteins) hold special promise for a wide range of clinical and biomedical applications in several disorders, including DR [12].

4. Oxidative stress and its role in the development of DR

Oxidative stress may be defined as an imbalance between the level of ROS or oxygen radicals and the antioxidant defenses in a biological system [10]. The term "ROS" includes all unstable metabolites of molecular oxygen (O_2) that have a higher reactivity than O_2, such as the superoxide radical ($O_2'^{\bullet}$) and the hydroxyl radical ($HO^{\bullet}$), and nonradical molecules, such as hydrogen peroxide (H_2O_2) [13]. To counteract the harmful effects of ROS, the cell has developed antioxidant defense mechanisms. Antioxidants may be classified according to their structure (enzymes or small nonenzymatic protein molecules) and antioxidants according to their source (endogenous or exogenous). There are many enzymes with an antioxidant role in the organism, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferases (GSTs), and the thioredoxin (Trx) system [14,15].

Increasing data indicate that oxidative stress is involved in the development of DR [16–19]. The retina has a high content of polyunsaturated fatty acids and has the highest oxygen uptake and glucose oxidation relative to any other tissue. This phenomenon renders the retina more susceptible to oxidative stress [20]. Oxidative stress-induced biochemical changes contribute to either functional or structural changes in the microvasculature in the retina [6]. Structural

changes range from basement membrane thickening and microvascular cell loss to capillary closure and acellular capillary formation [6]. ROS mediate these changes by both direct and indirect mechanisms. Structural changes may both contribute to and result from functional changes, such as altered blood flow, loss of intercellular junctions, and increased vessel permeability. Thus, oxidative stress-induced structural and functional changes appear to be highly interrelated in the pathogenesis of diabetic retinopathy (DR) [6].

Since long-term exposure to oxidative stress is strongly implicated in the pathogenesis of diabetic complications, polymorphic genes of detoxifying enzymes may be involved in the development of DR.

5. Polymorphisms in oxidative stress genes and risk for DR

Gene	Polymorphism		Relation to DR (significance level)	Population	Number of patients	Author	Year
MnSOD	rs4880	VV genotype (V16A)	Positive association ($p = 0.006$)	Slovenian	426	Petrovič et al.	2008
		AA genotype (V16A)	Positive association ($p = 0.03$)	Finnish	755	Kangas-Kontio et al.	2009
		C allele (C47T)	No significant association	Different ethnic origins	(17 articles) meta-analysis	Tian et al.	2011
		(V16A)	No significant association	North Indian	758	Vanita	2014
		AV genotype (V16A)	Positive association ($p < 0.0001$)	Northern Iranian	280	Haghighi et al.	2015
CAT	rs1001179	−262C/T	No significant association	Brazilian	520	Dos Santos et al.	2006
GPx	rs1050450	Pro197Leu	No study on DR	/	/	/	/
GSTM1, T1	Null genotype	GSTT1null	Positive association ($p = 0.01$)	Scottish	2015	Doney et al.	2005
		GSTT1null GSTM1null	Positive association ($p < 0.0001$) Positive association	Slovenian	604	Cilenšek et al.	2012

Gene	Polymorphism		Relation to DR (significance level)	Population	Number of patients	Author	Year
			(p = 0.01)				
		GSTM1null	Positive association (p = 0.04)	Iranian	115	Dadbinpour et al.	2013
		GSTM1null GSTT1null	No significant association	Iranian	404	Moasser et al.	2014
		GSTT1null	Positive association	Caucasian	3563 (meta-analysis)	Sun et al.	2015
GSTP1	rs947894	Ile105Val	No significant association	Slovenian	604	Cilenšek et al.	2012
		A313G	No significant association	Egyptian	105	Zaki et al.	2015
Trx	rs4485648	CT genotype (T9921C) TT genotype (T9921C)	Positive association (p = 0.028) Positive association (p = 0.026)	Slovenian	953	Mankoč Ramuš et al.	2015

Table 1. Genes affecting oxidative stress and diabetic retinopathy.

5.1. Manganese superoxide dismutase (MnSOD)

SOD catalyzes the breakdown of superoxide into H_2O_2 scavenging superoxide, and, because of its mitochondrial localization, MnSOD is considered as the first line of defense against oxidative stress [19]. A number of polymorphisms in the mitochondrial targeting sequence of *MnSOD* have been described, but only the A16V (C47T; rs4880) evokes functional consequences [21–24]. In fact, the alanine variant of MnSOD is thought to have an α-helical mitochondrial-targeting domain, whereas the valine variant of MnSOD appears to have a β-pleated sheet conformation. This conformational difference is thought to result in a more efficient transport of the alanine variant of MnSOD into mitochondria than the valine variant [25]. Thus, the valine variant has been associated with a 30–40% lower activity and an increased susceptibility to oxidative stress [22].

There are only a few studies indicating the association of the V16A polymorphism of the MnSOD gene with DR (**Table 1**). A meta-analysis comprising 17 studies, including type 1 and type 2 diabetic patients from different ethnic origins, implied that the C (Ala) allele of the C47T polymorphism in the MnSOD gene had a significant protective effect against microvascular

complications (DR an diabetic nephropathy), although the aforementioned C allele had no significant effect on the risk for DR alone [26]. Petrovič et al. have reported that the ValVal genotype of the Val16Ala polymorphism of the MnSOD might be a risk factor for DR [27]. In contrast, Kangas-Kontio et al. [24] could not confirm such association in their study, as they found a significantly higher frequency of the AlaAla genotype in diabetics (type 1 or type 2) with DR [24]. Furthermore, a study of northern Iranian T2DM patients revealed that the heterozygosity in codon 16 of the MnSOD is considered as a risk factor for DR in T2DM [28]. However, another study from north India did not confirm an association between this SNP and DR in T2DM patients [29].

These conflicting results may be due to the ethnical differences, different genetic backgrounds, and sizes of the study populations.

5.2. Catalase (CAT)

CAT is a potent scavenger of H_2O_2 and provides a powerful antioxidant defense in the retina. It prevents the formation of the more toxic hydroxyl radical ($HO^{\bullet}$) resulting from the reaction of H_2O_2 and ferrous ions [30]. In structures like the eye, a significant contribution of CAT to H_2O_2 detoxification was reported. The inhibition of catalase activity in the rabbit eyes increased the H_2O_2 concentration 2.5-fold, which was not compensated for by GPx activity [31].

It has been shown that genetic variations in the *CAT* gene and its promoter may play a role in a number of diseases associated with oxidative stress (e.g., atherosclerosis, hyperlipidemia, diabetes mellitus, hypertension, and neurodegenerative diseases) [32]. Although catalase is broadly studied, to the best of our knowledge, there is only one report in which no association was observed between the −262C/T polymorphism in the promoter region of the *CAT* gene and DR in Caucasian-Brazilian T2DM patients (**Table 1**) [33].

5.3. Glutathione peroxidases (GPxs)

GPxs are selenocysteine-containing enzymes that catalyze the reduction of H_2O_2 and lipid hydroperoxides to H_2O and lipid alcohols, respectively, in a reaction that utilizes reduced glutathione (GSH) as a reducing co-substrate. There are five known forms of GPx: cellular (GPx-1), gastrointestinal (GPx-2), plasma (GPx-3), phospholipid (GPx-4), and sperm (snGPx) [34]. The most abundant intracellular isoform is GPx-1; it is known as the classical or cytosolic antioxidant enzyme and is ubiquitously expressed. GPx-1 deficiency has been shown to promote endothelial dysfunction, heart failure, and abnormal structural changes in vasculature and myocardium [2,34].

GPx-1 has four SNPs that change the amino acid produced, but only one has been studied extensively in human disease [35]. This missense polymorphism changes the amino acid from proline (Pro) to leucine (Leu) at position 197 (rs1050450) and was associated with a reduction in transcription and enzyme activity of GPx-1 [36].

The GPx-1 Pro/Leu genotype has been linked to lung cancer, bladder cancer, and complications in T2DM. Studies assessing the association between GPx-1 Pro197Leu SNP genotypes and diabetes, stroke, brain tumors, and prostate cancer are inconclusive [35].

An abundance of GPx has been localized in the rabbit retina through immunohistochemistry [37]. However, to date, there has been no study to show the association between *GPx* gene polymorphisms and DR in T2DM patients (**Table 1**).

5.4. Glutathione S-transferases (GSTs)

The human glutathione S-transferases (GSTs) are a family of enzymes known to act in the body as a defense system for neutralizing free radicals. They play an important role in the detoxification of electrophiles by glutathione conjugation [38]. GST enzymes are coded by at least eight distinct loci: α (GSTA), μ (GSTM), θ (GSTT), π (GSTP), σ (GSTS), k (GSTK), o (GSTO), and τ (GSTZ), each containing one or more homodimeric or heterodimeric isoforms. Three loci in particular, GSTM1, GSTT1, and GSTP1, have received most of the attention. The GSTM1 locus has been mapped on chromosome 1p13.3, while the GSTT1 and GSTP1 loci can be found on chromosomes 22q11.2 and 11q13. Persons with homozygous deletions of either the GSTM1 or GSTT1 loci have no enzymatic functional activity of the respective enzyme [39,40]. A GSTP1 variant with a substitution in the active site of valine for isoleucine at codon 105 (Ile105Val) has a reduced ability to conjugate reactive electrophiles with glutathione and may therefore sensitize cells to free radical-mediated damage. The Val105 variant has been associated with susceptibility to smoking-related cancer and cardiovascular disease [41].

Numerous GST polymorphisms have been associated with an increased or decreased susceptibility to several diseases [39,42–45], but only a few studies examined the association of GST polymorphisms and DR in T2DM patients (**Table 1**). Cilenšek et al. proposed a protective effect for the GSTM1-null genotype against retinopathy [46], explained by an up-regulation of other antioxidant enzymes, such as MnSOD [47]. On the contrary, the result of the aforementioned study is inconsistent with the study that showed a significant correlation between the GSTM1-null genotype and DR [38]. The study carried out by Doney et al. demonstrated that GSTT1-null individuals have a more generalized vasculopathy with an increased risk of progression of both retinopathy and nephropathy [41]. These findings are in agreement with the reports by Cilenšek et al., who recently reported that individuals homozygous for the deletion of GSTT1 are at an ≈ 2-fold-greater risk of DR [46]. There is only one report suggesting that GST allelic variants are not associated with individual susceptibility to DR [48].

Since the results of studies were conflicting and inconclusive, Sun et al. [49] performed a meta-analysis. A total of five studies were included, all of which were conducted in Caucasians; one study used T1DM patients, while other studies used T2DM patients. They reported that an increased risk of DR was associated with the null genotype of GSTT1 and GSTT1 polymorphisms, respectively [49].

As regards the GSTP1 gene polymorphism (rs947894), the domination of the G allele results in the reduction of GSTP1 enzyme activity. Consequently, the cell becomes more susceptible

to mutation and damage from exposure to electrophiles and ROS [50]. Despite the significance noted in the G allele in the GSTP1 gene polymorphism among diabetic cases [51,52], two studies failed to demonstrate any significant association between the GSTP1 polymorphism and DR in T2DM patients [46,50].

5.5. Trx system

The Trx system is one of the central antioxidant systems in mammalian cells, maintaining a reducing environment by catalyzing electron flux from NADPH through Trx reductase to Trx, which reduces its target proteins using highly conserved thiol groups [53]. In mammals, both Trx and TrxR are expressed as dedicated isoforms for either predominantly cytosolic (Trx1 and TrxR1) or mitochondrial (Trx2 and TrxR2) localization [54].

Up-regulation of thioredoxin-interacting protein (TXNIP), an endogenous inhibitor of Trx, compromises cellular antioxidant and antiapoptotic defenses and stimulates pro-inflammatory cytokines expression [55]. Moreover, it is highly induced in the diabetic retina and plays a critical role in DR pathogenesis [56–59]. Mankoč Ramuš et al. searched for a connection between genetic variants within the mitochondrial Trx antioxidant defense system and DR (**Table 1**). The aforementioned study was the first to explore the association between seven single nucleotide polymorphisms (SNPs), including rs8140110, rs7211, rs7212, rs4755, rs1548357, rs4485648, and rs5748469, in the Trx2/TXNIP and TrxR2 genes, and the risk of DR in a case–control study of Slovenian patients with T2DM. They found an association between the rs4485648 polymorphism of the TrxR2 gene and DR in Caucasians with T2DM [60].

6. The role of epigenetics in the pathogenesis of diabetic retinopathy

The heritable, yet reversible changes in the gene expression that are independent of the order of the nucleotides within a gene are called epigenetic modifications. An organism's genome can be modified by naturally occurring ROS which are regularly produced as an inevitable by-product of the normal oxygen metabolism. Oxidative stress is defined as a condition associated with an aberrant increase in ROS generation in a cell.

In diabetes, oxidative stress is increased in the retina and its capillary cells and is considered as one of the major metabolic abnormalities associated with the development of DR [61–63]. Arguably, the resulting hyperglycemia-induced ROS production may also promote epigenetic alterations in DR. Fundamental epigenetic mechanisms include DNA cytosine methylation, histone post-translational modifications (PTMs) in the chromatin, and noncoding RNAs (ncRNAs), all of which can affect gene expression individually or cooperatively and modulate disease states [64].

DNA methylation is considered to be one of the most important modifications leading to disease [65]. In general, DNA methylation at 5′ cytosine of the CpG dinucleotides forms 5-methylated cytosine (5mC). The formation of 5mC in the promoter regions leads to gene

http://dx.doi.org/10.5772/63353

repression, whereas in genes bodies it might regulate transcription elongation and alternative splicing [66]. DNA methylation is brought about by DNA methyltransferases (Dnmts), and these enzymes use S-adenosyl methionine (SAM) as the methyl donor [67,68]. It is noteworthy that some studies have begun to uncover the role of DNA methylation in diabetes and its complications. In animal models, epigenetic silencing due to increased promoter DNA methylation has been linked to islet dysfunction and development of diabetes [69,70]. In a case-controlled study of 168 patients with type 2 DM, the global DNA methylation status was shown to be associated with DR. Additionally, the DNA methylation status exhibited a strong correlation with the progression of DR [71,72]. Apart from the increased activity of Dnmts in the retina and its capillary cells [73], histone-modifying machinery is also affected in diabetes.

In mammalian cells, chromosomal DNA is packed into chromatin, and chromatin is made up of subunits called nucleosomes. Each nucleosome consists of an octamer protein complex, containing two copies each of core histone proteins H2A, H2B, H3, and H4 with 147 bp of chromosomal DNA wrapped around it [74]. Despite such sophisticated DNA packaging, the N-terminal of histones remains vulnerable for PTMs and can be acetylated, methylated, and phosphorylated. Such epigenetic modifications alter the chromatin structure which subsequently affects the binding of transcription factors and can regulate the selective expression of genes in a particular tissue by acting as switches to control gene activity [75–79]. Acetylation, the most common histone modification, which is generally associated with gene activation, is regulated [80] by fine-tune between histone-acetylating and histone-deacetylating enzymes; histone acetyltransferases (HATs) add the acetyl group, while histone deacetylase (HDAC) removes the acetyl group. Histone K acetylation (Kac) is enzymatically mediated by HATs, such as p300, the CREB-binding protein (CBP), and the Tat-interactive protein 60 kDa (Tip60). In general, histone Kac (such as H3K9ac, H3K14ac, and H4K5ac) at gene promoters correlates with transcriptional activation, whereas its removal is associated with gene repression [81]. Experimental evidence using *in vitro* and *in vivo* models of DR has shown increased HDACs and decreased HAT activities and global acetylation [82]. However, Kadiyala et al. have discovered an increased histone acetylation [83]. The reason for the divergence of the published data is not yet known.

Histone methylation is the most complex modification, since its function depends on the precise methylation site and the degree of modification. Lysine residues can have up to three methylation sites, whereas arginines (R) can have up to two methylation sites [84]. Lysine methylation (Kme) is mediated by histone K methyltransferases (HMTs) and removed by K demethylases (KDMs) [85,86]. H3K4me1/2/3 and H3K36me2/3 are generally associated with transcriptionally active genome regions, whereas H3K9me3, H3K27me3, and H4K20me3 are related with repressed domains [81]. In the development of DR, superoxide levels are elevated in the retina, antioxidant defense system is compromised, MnSOD is inhibited, and mitochondria are swollen and dysfunctional [77,87–90]. Overexpression of MnSOD protects diabetes-induced mitochondrial damage and the development of DR [19,91]. Furthermore, *SOD2* is epigenetically modified with increased H4K20me3, H3K9ac, and p65 subunit of NF-kB at its promoter/enhancer [77]. Besides, Zhong and Kowluru revealed that the exposure of retinal capillary cells to high glucose decreases H3K4me at *SOD2* promoter and enhancer regions,

suggesting the role of H3K4 methylation in *SOD2* repression [78]. The possible mechanism for such decrease of methylation is the activation of lysine-specific demethylase-1 (LSD1).

Apart from histone epigenetic modifications, the role of ncRNA has evoked great interest because gene expression can vary due to the function of RNA molecules themselves as well as their interactions with DNA and/or proteins [92]. NcRNAs with less than 200 nucleotides are generally classified as short (i.e., microRNAs), while all larger transcripts are regarded as long ncRNA (lncRNA). There are several subtypes of long and short ncRNA species, many of which are involved in the regulation of gene expression, and these can be further grouped according to their genomic origins and biogenic processes [92]. Specifically, increasing emphasis is being placed on the ability of miRNAs and lncRNAs to regulate gene expression and modulate the actions of growth and inflammatory factors related to diabetic complications [64]. MicroRNAs are a class of highly conserved 19–25 nucleotide single-stranded ncRNAs that regulate gene expression at the posttranscriptional level [93,94]. They block gene translation via binding to complementary regions of the mRNA. Micro-RNAs are also able to initiate the degradation of mRNA strands to which they are bound [95]. Various recent reports have demonstrated alterations in miRNA expression in diabetic eyes. Subjects with proliferative and nonproliferative DR have different serum levels of miR-21, miR-181c, and miR-1179 [96]. Downregulation of miR-200b was observed in the retina in diabetes. In parallel, VEGF (target of miR-200b) mRNA and protein were elevated [97]. It is now becoming clear that oxidative stress causes the activation of the redox-sensitive transcription factors and altered expression of a number of genes, including *VEGF*. Under diabetic conditions, it acts to increase vascular permeability in the early stages of DR and fluid accumulates in the retinal tissue, causing macular oedema and exudate [98]. Up-regulation of miR-195 is shown to downregulate deacetylase Sirtuin 1(Sirt 1) [99], and in DR, the inhibition of Sirt 1 in the retina activates NF-kB, a redox-sensitive proapoptotic factor [100]. On the other hand, up-regulation of miR-29b exerted an antiapoptotic function in the retinal ganglion cells [101]. In addition, miRNAs are stable in biological fluids, such as urine and serum [64], in this view; miRNAs appear to represent valuable noninvasive biomarkers and a promising tool of new approaches for the treatment of DR.

LncRNAs participate in a variety of biological processes, such as chromosome imprinting, epigenetic regulation, cell-cycle control, cell apoptosis, and reprogramming of induced pluripotent stem cells [102,103]. Recently, a human β-cell transcriptome analysis had indicated that lncRNAs are dynamically regulated and abnormally expressed in type 2 diabetes [104]. The field of lncRNA research in ocular diseases is expanding rapidly. Notably, lncRNAs are involved in the pathogenesis of DR through the modulation of multiple pathogenetic pathways. Metastasis-associated lung adenocarcinoma transcript 1, a conserved lncRNA, may become a potential therapeutic target for the prognosis, diagnosis, and treatment of DR [105]. The following year, Yan et al. revealed a regulatory role of the lncRNA myocardial infarction-associated transcript (MIAT) in diabetes mellitus-induced microvascular dysfunction [106].

In the pathogenesis of DR, retinal mitochondria become dysfunctional, and capillary cell apoptosis precedes the development of retinal histopathology associated with DR [107–109]. Mitochondrial homeostasis is maintained by a close cooperation between nuclear DNA and

http://dx.doi.org/10.5772/63353

mitochondrial DNA (mtDNA) [110]. Due to the lack of supporting histones, and the close proximity to the superoxide-generating electron transport chain, mtDNA is prone to oxidative damage [111,112]. In diabetes, the activity of retinal Dnmts is increased, and the mtDNA replication enzyme, the polymerase γ-1 (*POLG1*) gene, is hypermethylated and its binding at the *D-loop* is impaired, resulting in decreased mtDNA biogenesis [73].

Lately, it is becoming apparent that small interfering RNAs ameliorated the hyperglycemia-induced decrease in mtDNA transcription and the increase in apoptosis. In fact, Mishra and Kowluru have recently discovered that modulation of Dnmt1 by pharmaceutical or molecular means could help maintain mitochondrial integrity and serve as a potential strategy to inhibit/halt the development of DR [113].

The "new antioxidant" concept represents the benefit of the consumption of fresh fruit and vegetables in diabetic patients. Research on foods of plant origin shows that they contain many non-nutritional compounds with an oxidative stress-protective effect (green tea, α-lipoic acid, carnitine, glucosinolates, carotenoids, epigallocatechin, flavonoids, resveratrol, etc.) [100,114–118]. It has been suggested that these compounds regulate free radical over-generation at the mitochondrial level, increase intracellular defenses, and secrete and activate detoxifying enzymes [100,118]. The same principles work for the aforementioned novel compounds.

7. Antioxidants and diabetic retinopathy

So far, oxidative stress has been demonstrated to play an important role in the development and progression of DR; therefore antioxidants are expected to be helpful in preventing DR and its progression [100,114–118]. Lipid peroxidation (LPO) is considered to be a major harmful consequence of ROS formation, as it reflects irreversible oxidative changes of membranes. Moreover, it must be emphasized that retinal cells are highly sensitive to oxidative damage caused by the constant photochemical reactions, and the high concentrations of polyunsaturated fatty acids that constitute their membranes are directly affected by LPO.

Several antioxidants have so far been considered helpful in terms of prevention of DR and its progression; however, only a few research groups demonstrated an important effect of antioxidants in a few ocular disorders, such as in macular degeneration and in DR [119,120].

The administration of combined antioxidant therapy is helpful by improving antioxidant capacity against ROS and protecting photoreceptors against radiation. Vitamins C and E act by normalizing numerous chemical reactions to diminish aging and degeneration caused by ROS. CAT is recommended in macular degeneration [119]. Just recently an interesting study has been reported to demonstrate the effect of an adjunctive antioxidant treatment in subjects with DR [120]. Either coenzyme Q10 (400 mg/day) or combined antioxidant therapy (composed of 10 mg of lutein, 4 mg of astaxanthin, 1 mg of zeaxanthin, 180 mg of vitamin C, 30 mg of vitamin E, 20 mg of zinc, and 1 mg of copper) proved to be effective and safe for improving the oxidative stress in DR [120]. Moreover, ingestion of coenzyme Q10 and combined antiox-

idant therapy was significantly superior for decreasing the LPO levels, to values closer to normal, an outcome similar to that reported recently in vitreous humor [120,121].

8. Pespectives

Changes in dietary habits and lifestyles associated with rapid economic growth have dramatically increased the incidence of diabetes and related chronic microvascular complications, i.e., DR. So far, several studies have demonstrated the importance of several environmental, genetic, and epigenetic factors. Modulation of epigenetic changes by pharmaceutical means may provide a potential strategy to retard the progression of DR. Besides intense medical management, these strategies include dietary measures and the introduction of epigenetic drugs, such as inhibitors of DNA methylation and histone demethylases. We presume that intense medical management may be especially helpful with subjects having increased genetic risk according to the findings of genetic studies.

To conclude, the impact of nutritional factors is still insufficiently understood for patients with DR and well-designed prospective randomized clinical trials are needed to address the role of nutritional factors, including antioxidants. Genetic biomarkers (DNA and RNA gene polymorphisms) may be especially helpful in risk prediction, prognosis, or prediction of response of DR on drugs or nutritional factors. Finally, personalized medicine will most probably have an important part in managing subjects at increased risk for DR according to clinical, genetic, and epigenetic information providing that genetic tests (i.e., cost) become more widely available and that the genetic markers will be confirmed in prospective studies.

Acknowledgements

The authors thank Mrs. Brina Beškovnik, BA, for revising the English.

Author details

Ines Cilenšek[1], Sara Mankoč Ramuš[1], Mojca Globočnik Petrovič[2] and Daniel Petrovič[1*]

*Address all correspondence to: daniel.petrovic@mf.uni-lj.si

1 Institute of Histology and Embryology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

2 University Medical Centre, Eye Clinic, Ljubljana, Slovenia

http://dx.doi.org/10.5772/63353

References

[1] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2012;35(Suppl 1):S64–S71.

[2] Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. Nat Rev Endocrinol. 2011; 8(4):228–36.

[3] Kowluru RA, Kowluru A, Mishra M, Kumar B. Oxidative stress and epigenetic modifications in the pathogenesis of diabetic retinopathy. Prog Retin Eye Res. 2015; 48:40–61

[4] Fong DS, Aiello L, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL 3rd, Klein R; American Diabetes Association. Diabetic retinopathy. Diabetes Care. 2003; 26(1):226–9.

[5] Nwanyanwu KH, Newman-Casey PA, Gardner TW, Lim JI. Beyond HbA1c: environmental risk factors for diabetic retinopathy. J Clin Exp Ophthalmol. 2015; 6:2.

[6] Madsen-Bouterse SA, Kowluru RA. Oxidative stress and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. Rev Endocr Metab Disord. 2008; 9(4):315–27.

[7] The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993; 329:977–86.

[8] UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998; 352:837–53.

[9] Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005; 54(6):1615–25.

[10] Tarr JM, Kaul K, Chopra M, Kohner EM, Chibber R. Pathophysiology of diabetic retinopathy. ISRN Ophthalmol. 2013; 2013:343560.

[11] Petrovič D. Candidate genes for proliferative diabetic retinopathy. Biomed Res Int. 2013; 2013:540416.

[12] https://www.broadinstitute.org/scientificcommunity/science/platforms/proteomics/biomarkers/.

[13] Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, Dhama K. Oxidative stress, prooxidants, and antioxidants: the interplay. Biomed Res Int. 2014; 2014:761264.

[14] Gürler B, Vural H, Yilmaz N, Oguz H, Satici A, Aksoy N. The role of oxidative stress in diabetic retinopathy. Eye (Lond). 2000; 14 Pt 5:730–5.

[15] Lu J, Holmgren A. The thioredoxin antioxidant system. Free Radic Biol Med. 2014; 66:75–87.

[16] Allen RG, Tresini M. Oxidative stress and gene regulation. Free Radic Biol Med. 2000; 28(3):463–99.

[17] Du Y, Miller CM, Kern TS. Hyperglycemia increases mitochondrial superoxide in retina and retinal cells. Free Radic Biol Med. 2003; 35(11):1491–9.

[18] Jain SK, McVie R, Duett J, Herbst JJ. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. Diabetes. 1989; 38(12):1539–43.

[19] Kowluru RA, Atasi L, Ho YS. Role of mitochondrial superoxide dismutase in the development of diabetic retinopathy. Invest Ophthalmol Vis Sci. 2006; 47(4):1594–9.

[20] Anderson RE, Rapp LM, Wiegand RD. Lipid peroxidation and retinal degeneration. Curr Eye Res. 1984; 3:223–7.

[21] Degoul F, Sutton A, Mansouri A, Cepanec C, Degott C, Fromenty B, Beaugrand M, Valla D. Homozygosity for alanine in the mitochondrial targeting sequence of superoxide dismutase and risk for severe alcoholic liver disease. Gastroenterology. 2001;120:1468–74.

[22] AQ: Please provide volume number for Ref. [22].Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagawa-Hattori Y, Shimizu Y, Mizuno Y. Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinsons disease. Biochem Biophys Res Commun. 1996; 561–5.

[23] Sutton A, Imbert A, Igoudjil A, Descatoire V, Cazanave S, Pessayre D, Degoul F. The manganese superoxide dismutase Ala16Val dimorphism modulates both mitochondrial import and mRNA stability. Pharmacogenet Genomics. 2005; 15:311–19.

[24] Kangas-Kontio T, Vavuli S, Kakko SJ, Penna J, Savolainen ER, Savolainen MJ, Liinamaa MJ. Polymorphism of the manganese superoxide dismutase gene but not of vascular endothelial growth factor gene is a risk factor for diabetic retinopathy. Br J Ophthalmol. 2009; 93(10):1401–6.

[25] Fujimoto H, Taguchi J, Imai Y, Ayabe S, Hashimoto H, Kobayashi H, Ogasawara K, Aizawa T, Yamakado M, Nagai R, Ohno M. Manganese superoxide dismutase polymorphism affects the oxidized low-density lipoprotein-induced apoptosis of macrophages and coronary artery disease. Eur Heart J. 2008; (10):1267–74.

[26] Tian C, Liu T, Fang S, Du X, Jia C. Association of C47T polymorphism in SOD2 gene with coronary artery disease: a case–control study and a meta-analysis. Mol Biol Rep. 2012; 39(5):5269–76.

[27] Petrovic MG, Cilensek I, Petrovic D. Manganese superoxide dismutase gene polymorphism (V16A) is associated with diabetic retinopathy in Slovene (Caucasians) type 2 diabetes patients. Dis Markers. 2008; 24(1):59–64.

[28] Haghighi SF, Salehi Z, Sabouri MR, Abbasi N. Polymorphic variant of MnSOD A16V and risk of diabetic retinopathy. Mol Biol (Mosk). 2015; 49(1):114–8.

[29] Vanita V. Association of RAGE (p.Gly82Ser) and MnSOD (p.Val16Ala) polymorphisms with diabetic retinopathy in T2DM patients from north India. Diabetes Res Clin Pract. 2014; 104(1):155–62.

[30] Chen B, Tang L. Protective effects of catalase on retinal ischemia/reperfusion injury in rats. Exp Eye Res. 2011; 93(5):599–606.

[31] Ohta Y, Yamasaki T, Niwa T, Niimi K, Majima Y, Ishiguro I. Role of catalase in retinal antioxidant defence system: its comparative study among rabbits, guinea pigs, and rats. Ophthalmic Res. 1996; 28(6):336–42.

[32] Kodydková J, Vávrová L, Kocík M, Žák A. Human catalase, its polymorphisms, regulation and changes of its activity in different diseases. Folia Biol (Praha). 2014; 60(4): 153–67.

[33] dos Santos KG, Canani LH, Gross JL, Tschiedel B, Souto KE, Roisenberg I. The catalase −262C/T promoter polymorphism and diabetic complications in Caucasians with type 2 diabetes. Dis Markers. 2006; 22(5–6):355–9.

[34] Banerjee M, Vats P. Reactive metabolites and antioxidant gene polymorphisms in Type 2 diabetes mellitus. Indian J Hum Genet. 2014; 20(1):10–9.

[35] Crawford A, Fassett RG, Geraghty DP, Kunde DA, Ball MJ, Robertson IK, Coombes JS. Relationships between single nucleotide polymorphisms of antioxidant enzymes and disease. Gene. 2012; 501(2):89–103.

[36] Matsuno S, Sasaki H, Yamasaki, Yamaoka H, Ogawa K, Nakatani M, Hamanishi T, Doi A, Nakano Y, Wakasaki H, Furuta H, Nishi M, Akamizu T, Nanjo K. Pro198Leu missense polymorphism of the glutathione peroxidase 1 gene might be a common genetic predisposition of distal symmetric polyneuropathy and macrovascular disease in Japanese type 2 diabetic patients. J Diabetes Investig. 2011; 2(6):474-82.

[37] Atalla LR, Sevanian A, Rao NA. Immunohistochemical localization of peroxidative enzymes in ocular tissue. CLAO J. 1990; 16(1 Suppl):S30–3.

[38] Dadbinpour A, Sheikhha MH, Darbouy M, Afkhami-Ardekani M. Investigating GSTT1 and GSTM1 null genotype as the risk factor of diabetes type 2 retinopathy. J Diabetes Metab Disord. 2013; 12(1):48.

[39] Mo Z, Gao Y, Cao Y, Gao F, Jian L. An updating meta-analysis of the GSTM1, GSTT1, and GSTP1 polymorphisms and prostate cancer: a HuGE review. Prostate. 2009; 69(6): 662–88.

[40] Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM, Ketterer B, Taylor JB. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J. 1994; 300 (Pt 1):271–6.

[41] Doney AS, Lee S, Leese GP, Morris AD, Palmer CN. Increased cardiovascular morbidity and mortality in type 2 diabetes is associated with the glutathione S transferase theta-null genotype: a Go-DARTS study. Circulation. 2005; 111(22):2927–34.

[42] Stoian A, Bănescu C, Bălaşa RI, Moţăţăianu A, Stoian M, Moldovan VG, Voidăzan S, Dobreanu M. Influence of GSTM1, GSTT1, and GSTP1 polymorphisms on type 2 diabetes mellitus and diabetic sensorimotor peripheral neuropathy risk. Dis Markers. 2015; 2015:638693.

[43] Petrovič D, Peterlin B. GSTM1-null and GSTT1-null genotypes are associated with essential arterial hypertension in patients with type 2 diabetes. Clin Biochem. 2014; 47(7–8):574–7.

[44] Safarinejad MR, Safarinejad S, Shafiei N, Safarinejad S. Association of genetic polymorphism of glutathione S-transferase (GSTM1, GSTT1, GSTP1) with bladder cancer susceptibility. Urol Oncol. 2013; 31(7):1193–203.

[45] Boccia S, La Torre G, Gianfagna F, Mannocci A, Ricciardi G. Glutathione S-transferase T1 status and gastric cancer risk: a meta-analysis of the literature. Mutagenesis. 2006; 21(2):115–23.

[46] Cilenšek I, Mankoč S, Petrovič MG, Petrovič D. GSTT1 null genotype is a risk factor for diabetic retinopathy in Caucasians with type 2 diabetes, whereas GSTM1 null genotype might confer protection against retinopathy. Dis Markers. 2012; 32(2):93–9.

[47] Otto-Knapp R, Jurgovsky K, Schierhorn K, Kunkel G. Antioxidative enzymes in human nasal mucosa after exposure to ozone. Possible role of GSTM1 deficiency. Inflamm Res. 2003; 52(2):51–5.

[48] Moasser E, Azarpira N, Shirazi B, Saadat M, Geramizadeh B. Genetic polymorphisms of glutathione-S-transferase M1 and T1 genes with risk of diabetic retinopathy in Iranian population. Iran J Basic Med Sci. 2014; 17(5):351–6.

[49] Sun L, Zhang Y, Xiong Y. GSTM1 and GSTT1 null genotype and diabetic retinopathy: a meta-analysis. Int J Clin Exp Med. 2015; 8(2):1677–83.

[50] Zaki MA, Moghazy TF, El-Deeb MMK, Mohamed AH, Mohamed NAA. Glutathione S-transferase M1, T1 and P1 gene polymorphisms and the risk of developing type 2 diabetes mellitus in Egyptian diabetic patients with and without diabetic vascular complications. Alexandria J Med. 2015; 51(1):73–82.

[51] Bid HK, Konwar R, Saxena M, Chaudhari P, Agrawal CG, Banerjee M. Association of glutathione S-transferase (GSTM1, T1 and P1) gene polymorphisms with type 2 diabetes mellitus in north Indian population. J Postgrad Med. 2010; 56(3):176–81.

[52] Amer MA, Ghattas MH, Abo-Elmatty DM, Abou-El-Ela SH. Evaluation of glutathione S-transferase P1 genetic variants affecting type-2 diabetes susceptibility and glycemic control. Arch Med Sci. 2012; 8(4):631–6.

[53] Lee S, Kim SM, Lee RT. Thioredoxin and thioredoxin target proteins: from molecular mechanisms to functional significance. Antioxid Redox Signal. 2013; 18(10):1165–207.

[54] Arnér ES, Holmgren A. The thioredoxin system in cancer. Semin Cancer Biol. 2006; 16(6):420–6.

[55] Al-Gayyar MM, Abdelsaid MA, Matragoon S, Pillai BA, El-Remessy AB. Thioredoxin interacting protein is a novel mediator of retinal inflammation and neurotoxicity. Br J Pharmacol. 2011; 164(1):170–80.

[56] Devi TS, Lee I, Hüttemann M, Kumar A, Nantwi KD, Singh LP. TXNIP links innate host defense mechanisms to oxidative stress and inflammation in retinal Muller glia under chronic hyperglycemia: implications for diabetic retinopathy. Exp Diabetes Res. 2012; 2012:438238.

[57] Devi TS, Hosoya K, Terasaki T, Singh LP. Critical role of TXNIP in oxidative stress, DNA damage and retinal pericyte apoptosis under high glucose: implications for diabetic retinopathy. Exp Cell Res. 2013; 319(7):1001–12.

[58] Perrone L, Devi TS, Hosoya K, Terasaki T, Singh LP. Thioredoxin interacting protein (TXNIP) induces inflammation through chromatin modification in retinal capillary endothelial cells under diabetic conditions. J Cell Physiol. 2009; 221(1):262–72.

[59] Perrone L, Devi TS, Hosoya KI, Terasaki T, Singh LP. Inhibition of TXNIP expression in vivo blocks early pathologies of diabetic retinopathy. Cell Death Dis. 2010; 1:e65.

[60] Ramus SM, Cilensek I, Petrovic MG, Soucek M, Kruzliak P, Petrovic D. Single nucleotide polymorphisms in the Trx2/TXNIP and TrxR2 genes of the mitochondrial thioredoxin antioxidant system and the risk of diabetic retinopathy in patients with Type 2 diabetes mellitus. J Diabetes Complications. 2015; pii: S1056-8727(15)00459-6.

[61] Kowluru RA. Effect of reinstitution of good glycemic control on retinal oxidative stress and nitrative stress in diabetic rats. Diabetes. 2003; 52(3):818–23.

[62] Kowluru RA, Engerman RL, Kern TS. Abnormalities of retinal metabolism in diabetes or experimental galactosemia. VI. Comparison of retinal and cerebral cortex metabolism, and effects of antioxidant therapy. Free Radic Biol Med. 1999; 26(3–4):371–8.

[63] Kowluru RA, Tang J, Kern TS. Abnormalities of retinal metabolism in diabetes and experimental galactosemia. VII. Effect of long-term administration of antioxidants on the development of retinopathy. Diabetes. 2001; 50(8):1938–42.

[64] Kato M, Natarajan R. Diabetic nephropathy—emerging epigenetic mechanisms. Nat Rev Nephrol. 2014; 10(9):517–30.

[65] Rasool M, Malik A, Naseer MI, Manan A, Ansari S, Begum I, Qazi MH, Pushparaj P, Abuzenadah AM, Al-Qahtani MH, Kamal MA, Gan S. The role of epigenetics in

personalized medicine: challenges and opportunities. BMC Med Genomics. 2015; 8 (Suppl 1):S5.

[66] Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet. 2012; 13(7):484–92.

[67] Deaton AM, Bird A. CpG islands and the regulation of transcription. Genes Dev. 2011; 25(10):1010–22.

[68] Majumdar S, Buckles E, Estrada J, Koochekpour S. Aberrant DNA methylation and prostate cancer. Curr Genomics. 2011; 12(7):486–505.

[69] Ling C, Del Guerra S, Lupi R, Rönn T, Granhall C, Luthman H, Masiello P,Marchetti P, Groop L, Del Prato S. Epigenetic regulation of PPARGC1A in human type 2 diabetic islets and effect on insulin secretion. Diabetologia. 2008; 51(4):615–22.

[70] Park JH, Stoffers DA, Nicholls RD, Simmons RA. Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of Pdx1. J Clin Invest. 2008; 118(6):2316–24.

[71] Maghbooli Z, Larijani B, Emamgholipour S, Amini M, Keshtkar A, Pasalar P. Aberrant DNA methylation patterns in diabetic nephropathy. J Diabetes Metab Disord. 2014; 13:69.

[72] Maghbooli Z, Hossein-nezhad A, Larijani B, Amini M, Keshtkar A. Global DNA methylation as a possible biomarker for diabetic retinopathy. Diabetes Metab Res Rev. 2015; 31(2):183–9.

[73] Tewari S, Zhong Q, Santos JM, Kowluru RA. Mitochondria DNA replication and DNA methylation in the metabolic memory associated with continued progression of diabetic retinopathy. Invest Ophthalmol Vis Sci. 2012; 53(8):4881–8.

[74] Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature. 1997; 389(6648):251–60.

[75] Berger SL. The complex language of chromatin regulation during transcription. Nature. 2007; 447(7143):407–12.

[76] Vaissière T, Sawan C, Herceg Z. Epigenetic interplay between histone modifications and DNA methylation in gene silencing. Mutat Res. 2008; 659(1–2):40–8.

[77] Zhong Q, Kowluru RA. Epigenetic changes in mitochondrial superoxide dismutase in the retina and the development of diabetic retinopathy. Diabetes. 2011; 60(4):1304–13.

[78] Zhong Q, Kowluru RA. Epigenetic modification of Sod2 in the development of diabetic retinopathy and in the metabolic memory: role of histone methylation. Invest Ophthalmol Vis Sci. 2013; 54(1):244–50.

[79] Zhong Q, Kowluru RA. Regulation of matrix metalloproteinase-9 by epigenetic modifications and the development of diabetic retinopathy. Diabetes. 2013; 62(7):2559–68.

[80] Glozak MA, Seto E. Histone deacetylases and cancer. Oncogene. 2007; 26(37):5420–32.

[81] Kouzarides T. Chromatin modifications and their function. Cell. 2007; 128(4):693–705.

[82] Zhong Q, Kowluru RA. Role of histone acetylation in the development of diabetic retinopathy and the metabolic memory phenomenon. J Cell Biochem. 2010; 110(6): 1306–13.

[83] Kadiyala CS, Zheng L, Du Y, Yohannes E, Kao HY, Miyagi M, Kern TS. Acetylation of retinal histones in diabetes increases inflammatory proteins: effects of minocycline and manipulation of histone acetyltransferase (HAT) and histone deacetylase (HDAC). J Biol Chem. 2012; 287(31):25869–80.

[84] Izzo A, Schneider R. Chatting histone modifications in mammals. Brief Funct Genomics. 2010; 9(5–6):429–43.

[85] Klose RJ, Zhang Y. Regulation of histone methylation by demethylimination and demethylation. Nat Rev Mol Cell Biol. 2007; 8(4):307–18.

[86] Zhou VW, Goren A, Bernstein BE. Charting histone modifications and the functional organization of mammalian genomes. Nat Rev Genet. 2011; 12(1):7–18.

[87] Kowluru RA, Abbas SN. Diabetes-induced mitochondrial dysfunction in the retina. Invest Ophthalmol Vis Sci. 2003; 44(12):5327–34.

[88] Kowluru RA, Kowluru V, Xiong Y, Ho YS. Overexpression of mitochondrial superoxide dismutase in mice protects the retina from diabetes-induced oxidative stress. Free Radic Biol Med. 2006; 41(8):1191–6.

[89] Madsen-Bouterse SA, Mohammad G, Kanwar M, Kowluru RA. Role of mitochondrial DNA damage in the development of diabetic retinopathy, and the metabolic memory phenomenon associated with its progression. Antioxid Redox Signal. 2010; 13(6):797–805.

[90] Zhong Q, Kowluru RA. Diabetic retinopathy and damage to mitochondrial structure and transport machinery. Invest Ophthalmol Vis Sci. 2011; 52(12):8739–46.

[91] Kanwar M, Chan PS, Kern TS, Kowluru RA. Oxidative damage in the retinal mitochondria of diabetic mice: possible protection by superoxide dismutase. Invest Ophthalmol Vis Sci. 2007; 48(8):3805–11.

[92] Peschansky VJ, Wahlestedt C. Non-coding RNAs as direct and indirect modulators of epigenetic regulation. Epigenetics. 2014; 9(1):3–12.

[93] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116(2):281–97.

[94] Plasterk RH. Micro RNAs in animal development. Cell. 2006; 124(5):877–81.

[95] Wutz A. RNA-mediated silencing mechanisms in mammalian cells. Prog Mol Biol Transl Sci. 2011; 101:351–76.

[96] Qing S, Yuan S, Yun C, Hui H, Mao P, Wen F, Ding Y, Liu Q. Serum miRNA biomarkers serve as a fingerprint for proliferative diabetic retinopathy. Cell Physiol Biochem. 2014; 34(5):1733–40.

[97] McArthur K, Feng B, Wu Y, Chen S, Chakrabarti S. MicroRNA-200b regulates vascular endothelial growth factor-mediated alterations in diabetic retinopathy. Diabetes. 2011; 60(4):1314–23.

[98] Cukiernik M, Hileeto D, Evans T, Mukherjee S, Downey D, Chakrabarti S. Vascular endothelial growth factor in diabetes induced early retinal abnormalities. Diabetes Res Clin Pract. 2004; 65(3):197–208.

[99] Chen Z, Shentu TP, Wen L, Johnson DA, Shyy JY. Regulation of SIRT1 by oxidative stress-responsive miRNAs and a systematic approach to identify its role in the endothelium. Antioxid Redox Signal. 2013; 19(13):1522–38.

[100] Kowluru RA, Santos JM, Zhong Q. Sirt1, a negative regulator of matrix metalloproteinase-9 in diabetic retinopathy. Invest Ophthalmol Vis Sci. 2014; 55(9):5653–60.

[101] Silva VA, Polesskaya A, Sousa TA, Corrêa VM, André ND, Reis RI, Kettelhut IC, Harel-Bellan A, De Lucca FL. Expression and cellular localization of microRNA-29b and RAX, an activator of the RNA-dependent protein kinase (PKR), in the retina of streptozotocin-induced diabetic rats. Mol Vis. 2011; 17:2228–40.

[102] Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet. 2009; 10(3):155–9.

[103] Wapinski O, Chang HY. Long noncoding RNAs and human disease. Trends Cell Biol. 2011; 21(6):354–61.

[104] Morán I, Akerman I, van de Bunt M, Xie R, Benazra M, Nammo T, Arnes L, Nakić N, García-Hurtado J, Rodríguez-Seguí S, Pasquali L, Sauty-Colace C, Beucher A, Scharfmann R, van Arensbergen J, Johnson PR, Berry A, Lee C, Harkins T, Gmyr V, Pattou F, Kerr-Conte J, Piemonti L, Berney T, Hanley N, Gloyn AL, Sussel L, Langman L, Brayman KL, Sander M, McCarthy MI, Ravassard P, Ferrer J. Human β cell transcriptome analysis uncovers lncRNAs that are tissue-specific, dynamically regulated, and abnormally expressed in type 2 diabetes. Cell Metab. 2012; 16(4):435–48.

[105] Yan B, Tao ZF, Li XM, Zhang H, Yao J, Jiang Q. Aberrant expression of long noncoding RNAs in early diabetic retinopathy. Invest Ophthalmol Vis Sci. 2014; 55(2):941–51.

[106] Yan B, Yao J, Liu JY, Li XM, Wang XQ, Li YJ, Tao ZF, Song YC, Chen Q, Jiang Q. lncRNA-MIAT regulates microvascular dysfunction by functioning as a competing endogenous RNA. Circ Res. 2015; 116(7):1143–56.

[107] Kern TS, Tang J, Mizutani M, Kowluru RA, Nagaraj RH, Romeo G, Podesta F, Lorenzi M. Response of capillary cell death to aminoguanidine predicts the development of retinopathy: comparison of diabetes and galactosemia. Invest Ophthalmol Vis Sci. 2000; 41(12):3972–8.

[108] Kowluru RA. Mitochondria damage in the pathogenesis of diabetic retinopathy and in the metabolic memory associated with its continued progression. Curr Med Chem. 2013; 20(26):3226–33.

[109] Kowluru RA, Chan PS. Metabolic memory in diabetes—from in vitro oddity to in vivo problem: role of apoptosis. Brain Res Bull. 2010; 81(2–3):297–302.

[110] Scarpulla RC. Nucleus-encoded regulators of mitochondrial function: integration of respiratory chain expression, nutrient sensing and metabolic stress. Biochim Biophys Acta. 2012; 1819(9–10):1088–97.

[111] Alexeyev M, Shokolenko I, Wilson G, LeDoux S. The maintenance of mitochondrial DNA integrity—critical analysis and update. Cold Spring Harb Perspect Biol. 2013; 5(5):a012641.

[112] Shokolenko I, Venediktova N, Bochkareva A, Wilson GL, Alexeyev MF. Oxidative stress induces degradation of mitochondrial DNA. Nucleic Acids Res. 2009; 37(8):2539–48.

[113] Mishra M, Kowluru RA. Epigenetic modification of mitochondrial DNA in the development of diabetic retinopathy. Invest Ophthalmol Vis Sci. 2015; 56(9):5133–42.

[114] Cao Y, Cao R. Angiogenesis inhibited by drinking tea. Nature. 1999;398(6726):381.

[115] Chan CM, Chang HH, Wang VC, Huang CL, Hung CF. Inhibitory effects of resveratrol on PDGF-BB-induced retinal pigment epithelial cell migration via PDGFRβ, PI3K/Akt and MAPK pathways. PLoS One. 2013; 8(2):e56819.

[116] Alqahtani A, Hamid K, Kam A, Wong KH, Abdelhak Z, Razmovski-Naumovski V, Chan K, Li KM, Groundwater PW, Li GQ. The pentacyclic triterpenoids in herbal medicines and their pharmacological activities in diabetes and diabetic complications. Curr Med Chem. 2013; 20(7):908–31.

[117] Please provide the volume number and page range for Ref. [117].Zeng K, Yang N, Wang D, Li S, Ming J, Wang J, Yu X, Song Y, Zhou X, Yang Y. Resveratrol prevents retinal dysfunction by regulating glutamate transporters, glutamine synthetase expression and activity in diabetic retina. Neurochem Res. 2015 Dec 16 [Epub ahead of print].

[118] Ulker E, Parker WH, Raj A, Qu ZC, May JM. Ascorbic acid prevents VEGF-induced increases in endothelial barrier permeability. Mol Cell Biochem. 2016; 412(1–2):73–9.

[119] Parisi V, Tedeschi M, Gallinaro G, Varano M, Saviano S, Piermarocchi S, CARMIS Study Group. Carotenoids and anti- oxidants in age-related maculopathy Italian study:

multifocal electroretinogram modifications after 1 year. Ophthalmology. 2008; 115(2): 324–33.

[120] Rodríguez-Carrizalez AD, Castellanos-González JA, Martínez-Romero EC, Miller-Arrevillaga G, Pacheco-Moisés FP, Román-Pintos LM, Miranda-Díaz AG. The effect of ubiquinone and combined antioxidant therapy on oxidative stress markers in non-proliferative diabetic retinopathy: a phase IIa, randomized, double-blind, and placebo-controlled study. Redox Rep. 2015 Aug 31 [Epub ahead of print].

[121] Mancino R, Di Pierro D, Varesi C, Cerulli A, Feraco A, Cedrone C, et al. Lipid peroxidation and total antioxidant capacity in vitreous, aqueous humor, and blood samples from patients with diabetic retinopathy. Mol Vis. 2011; 17:1298–304.

IntechOpen