Epigenetic Insights: DNA Methylation and Gene Expression in Diabetic Retinopathy–A Mini Review

Shaista[1], Abdul Waris[1*], Sheelu Shafiq Siddiqi[2], Tanusree Debbarman[3], Vivek Ram K.U.[1], Dimple Thacker[1]

[1]Junior Resident, Aligarh Muslim University Institute of Ophthalmology, J.N. Medical College and Hospital, Aligarh Muslim University, Aligarh, UP 202002, India

[2]Director and Professor, Rajiv Gandhi Centre for Diabetes and Endocrinology, J.N. Medical College and Hospital, Aligarh Muslim University, Aligarh, UP 202002, India

[3]Research Associate, Rajiv Gandhi Centre for Diabetes and Endocrinology, J.N. Medical College and Hospital, Aligarh Muslim University, Aligarh, UP 202002, India

Corresponding Author*

Abdul Waris

Professor, Aligarh Muslim University Institute of Ophthalmology,

J.N. Medical College and Hospital,

Aligarh Muslim University,

Aligarh, UP 202002, India

E-mail: warisabduleye@gmail.com

Received: October 14, 2024, Manuscript No. JDM-24-34587; **Editor assigned:** October 16, 2024, PreQC No. JDM-24-34587 (PQ); **Reviewed:** October 30, 2024, QC No. JDM-24-34587; **Revised:** November 21, 2024, Manuscript No. JDM-24-34587 (R); **Published:** November 28, 2024, DOI: 10.35248/2155-6156.24.15(11).1178

Abstract

Background: Diabetic Retinopathy (DR) ranks among the primary causes of vision loss in both developing and developed nations. Its development, pathophysiology, and prognosis have been linked to genetics, epigenetics, and environmental factors. Epigenetics have been linked to normal physiological foetal development during embryogenesis, cancer, psychiatric disorders, cardiovascular diseases, renovascular diseases, cerebrovascular diseases, etc. Diabetes, being one of the diseases that cause multisystem disorders, retinopathy being one of them, has been closely associated with epigenetics, especially DNA methylation in its development.

Overview and methodology: Our review article focuses on the existing literature on epigenetics, mainly methylation. It discusses the current understanding of global DNA methylation in pathogenesis of diabetic retinopathy and how it can be utilised for early diagnosis and treatment in the early phases of DR. The article compiles existing knowledge about the role of global DNA methylation in the development of diabetic retinopathy and its potential applications in diagnosing and treating the condition during its early stages. We have gathered our information from various articles published in PubMed, google scholar, Cochrane library and research gate. This article will upgrade our knowledge of DNA methylation in diabetic retinopathy and its possible use as a biomarker for early diagnosis in the near future. It will undoubtedly provide new hope for people prone to developing retinopathy and for people suffering from diabetic retinopathy.

Results: Based on an epigenetic study done in middle east population 168 patients (74 patients with retinopathy and 94 patients without retinopathy) using reversed phase high pressure liquid chromatography of peripheral blood leukocytes to determine 5-methylcytosine content of whole DNA status in two groups, either with or without retinopathy and concluded that methylation levels were significantly higher in diabetic retinopathy patients and a positive correlation between the whole DNA methylation levels and progression of diabetic retinopathy.

Conclusion: A need for more extensive research involving multiple centres and diverse racial backgrounds is crucial in studying diabetic individuals with existing retinopathy, those at risk of developing retinopathy, and even healthy individuals prone to diabetes. These types of studies would be essential for determining the viability of methylation as a biomarker and potential therapeutic target in these populations.

Keywords: Diabetic Retinopathy (DR) • Epigenetics • DNA methylation

Introduction

Epigenetics is change in gene function caused by change in chromosomes without DNA sequence changes. They are stable, static throughout life, hereditable, influenced by environment, lifestyle and disease process. There are three types of epigenetic modification: Methylation, histone modification, non-coding RNA expression. These systems have the ability to independently control particular epigenetic phenomena, but they can also work together to cooperatively control several physiological processes.

DNA methylation is the process of transfer of methyl group to the C-5 position of the cytosine ring by an enzyme DNA Methyltransferase (DNMT) [1]. 98% of DNA methylation in human occur in CpG dinucleotide in somatic cells while some occurs in embryonic cells (Figure 1) [2]. It's involvement in genomic imprinting, X chromosome inactivation, and the suppression of repetitive elements is critical for normal development and proper cellular functioning. Furthermore, dysregulation of DNA methylation has been connected to a number of illnesses [3].

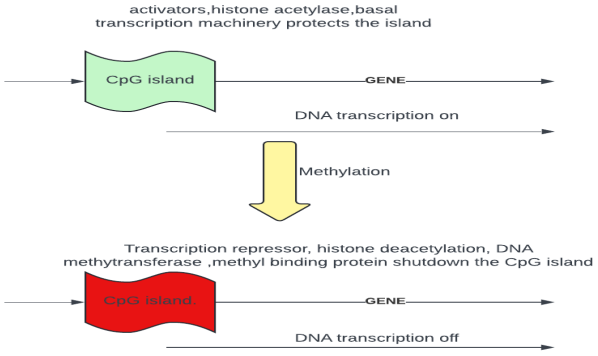

Figure 1. Effect of DNA methylation on DNA transcription (CpG: High concentration base pair of cytosine guanine nucleotide).

There are three types of DNMT [4]. DNMT 1 is in charge of transmitting the daughter strand's copy of the DNA methylation pattern during DNA replication during S phase of the cell cycle [5]. DNMT 2 instead of methylating DNA, methylates cytosine in the aspartic acid anticodon loop of t-RNA [6]. DNMT 3A AND 3B perform methylation *de novo* during development. Mice lacking DNMT 3A dies at 4 week of age and DNMT 3B knock out becomes lethal at embryonic age of E 14.5 to E 18.5 [7]. DNMT 3L helps in *de novo* methyl transfer by increasing the ability of DNA to bind methyl donor [8]. DNMT 3L null mice are viable but heterozygous mice derived from homozygous DNMT null oocyte dies around E 9. In conclusion, DNMT 1 is necessary for *de novo* methylation of genomic DNA, and

DNMT 3A AND 3B support replication-related maintenance methylation [9].

Literature Review

DNA methylation and diabetes retinopathy

In diabetics, the retina's vasculature, DNMT expression is elevated and DNMT is activated. Transcription is impacted by CpG hypermethylation because it prevents certain transcription factors from attaching to the DNA sequence [10]. CG dinucleotide within promoter is protected from methylation. Hypermethylation of CpG dinucleotide recruits Methylated CpG (MeCP) binding protein to bind the promoter region causing repression of transcription [11].

The TET dioxygenase family, consisting of TET1, TET2, and TET3, is essential for DNA demethylation as it transforms 5-methylcytosine into 5-hydroxymethylcytosine [12]. Due to this, certain genes can be activated and expressed and the chromatin can be opened up for transcription factor binding [13]. In people with diabetes, Tets are also activated in the retina and its vascular system. TET2 is the most extensively researched and closely connected subtype in the pathophysiology of DR [14]. Age-related Macular Degeneration (AMD), retinitis pigmentosa, and DR are linked to DNA methylation (Figure 2) [15].

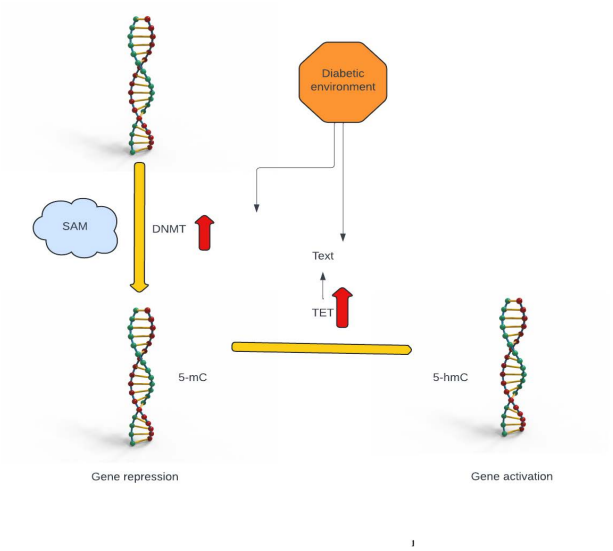

Figure 2. Increased expression on DNMT and TET causing gene expression and repression respectively.

Note: SAM: S-Adenosyl Methionine; DNMT: DNA Methyltrasferases; 5-mC: 5-Methylcystosine; 5-hmC: 5-Hydroxy Methylcytosine; TET: 10.11 Translocase

Through three additional pathways—oxidative stress, inflammation, and neovascularization—DNA methylation also plays a role in the pathogenic process of DR.

Oxidative stress

The term "oxidative stress" describes the harmful impact that Reactive Oxygen Species (ROS) can have on tissues, cells, or organs [16]. High levels of ROS can harm lipids, membranes, proteins, and nucleotides, among other cell components. The retina is an excellent target for Reactive Oxygen Species (ROS) due to its high oxygen consumption and metabolic rate. Diabetes-induced cytosolic ROS damage the retina's mitochondria and speed up the death of retinal capillary cells, which is an early phase in the retinal vascular system that leads to Diabetic Retinopathy (DR).

By possibly contributing to the development of DR through oxidative stress phenomenon, aberrant DNA methylation in the following linked genes may be the mechanism.

Matrix Metalloproteinase-9 (MMP-9): The transcription of MMP-9, an enzyme linked to retinal mitochondrial injury, is regulated by the methylation state of its DNA. Hyperglycaemia enhances Dnmt1 and TET2 binding to the *MMP-9* gene promoter in retinal endothelial cells. After Dnmt1 adds a methyl group to the cytosine to generate 5Mc, TET2 demethylates the cytosine into 5hmC, which activates MMP-9 transcription. Lastly, elevated *MMP-9* gene transcription and activity damages retinal mitochondria and encourage oxidative stress, which may result in the onset of DR [17].

mtDNA-related methylation: POLG1, D-loop, and MLH1: Diabetes causes malfunction in the retina's mitochondria, which damages the DNA (mtDNA) and increases the rate at which capillary cells die. These problems appear prior to any discernible histological alterations, suggesting a major role for mitochondria in the development of diabetic retinopathy [18]. Furthermore, a dysfunctional Electron Transport Chain (ETC) system affects the effective electron transfer that occurs during oxidative phosphorylation and produces Reactive Oxygen Species (ROS). This creates a positive feedback loop wherein ROS stimulate oxidative stress and subsequently produce additional ROS. Ultimately, the process of apoptosis is started when too much cytochrome c from damaged mitochondria seeps into the cytoplasm [19].

DNA methylation, which is frequently seen in Nuclear DNA (nDNA), is also seen in mitochondrial DNA (mtDNA), which has Tet and Dnmt sequences. Diabetes results in the activation of Dnmts expression, hypermethylation of mtDNA in retinal mitochondria, impaired transcription of mtDNA-encoded genes, defective mitochondria, and an acceleration of capillary cell apoptosis. The link between nDNA and mtDNA maintains mitochondrial homeostasis, and mutations in nuclear gene expression brought on by abnormal DNA methylation can jeopardise mitochondrial function. The catalytic subunit of the nuclear-encoded enzyme responsible for mtDNA replication is called Polymerase Gamma 1 (*POLG1)* [20].

"In addition to binding *POLG1* with the Displacement loop (*D-loop*) to regulate its replication, POLG is involved in repairing mtDNA damage. Due to retinal Dnmts, which are activated in DM, the regulatory site of *POLG1* is hypermethylated. This hypermethylation impairs mtDNA biogenesis and its transcription and damages the ETC system, speeding up the deleterious loop of ROS. The *D-loop* region is a critical control point for mtDNA transcription and replication. Compared with other regions of mtDNA in DM, the *D-loop* is more highly methylated and disrupts the transcription of mtDNA-encoded genes that are indispensable for maintaining the ETC system, which increases the electron leakage of the ETC complexes. These harmful results directly produce excessive superoxide radicals, aggravating the development of DR. In addition, the D-loop is the site where mtDNA binds to the inner mitochondrial membrane and is more vulnerable to damage than other regions of mtDNA.

In addition, the status of mtDNA methylation is positively correlated with increased base mismatches. MutL Homolog 1 (*MLH1*) is an enzyme important in the DNA Mismatch Repair (MMR) pathway" [21]. Hyperglycaemia conditions reduce MLH1 mitochondrial localization and hyper-methylate its promoter with activated Dnmt1. Finally, the repression of gene transcripts leads to mtDNA mismatches and mitochondrial damage. In conclusion, the aforementioned research indicates that maintaining a state of equilibrium in DNA methylation might have an impact on the prevention of mitochondrial DNA damage and the deceleration or obstruction of retinal disease progression.

Ras-related C3 botulinum toxin substrate 1 (RAC1): RAC1 is an essential component of NADPH Oxidases (Nox), which is an early phase in the formation of DR in which the majority of cytosolic ROS are produced by Noxs in retinal cells. Functional and transcriptional activation of *RAC1* in the retina of DM is associated with the promoter's DNA methylation conditions. Although Dnmt1 produces 5mC at the *RAC1* promoter when Dnmts and Tets are both activated in DM, a simultaneous rise in TET2 hydroxy methylates it to 5hmC, initiating the transcription of RAC1. This finding stimulates capillary cell apoptosis and Nox2-ROS-mitochondrial damage, which ultimately results in DR [22].

Peroxisome Proliferator-Activated Receptor Alpha (PPARα): PPARα functions as a transcription factor and contributes to the control of oxidative stress. Dnmt1 is highly expressed and interacts with the PPARα promoter to enhance its DNA methylation levels and suppress PPARα expression in Human Retinal Capillary Pericytes (HRCPs) treated with high hyperglycemia. As a result, ROS levels and the quantity of apoptotic cells are sharply increased. Furthermore, PPARα methylation can be inhibited and retinal cell death can be decreased by Dnmts suppression, according to a recent study. These provide compelling proof that Dnmt1-mediated PPARα DNA methylation speeds up ROS production and death, leading to a rise in defective retinal cells [23].

Mitochondrial Superoxide Dismutase (MnSOD): MnSOD is in charge of eliminating mitochondrial malfunction and scavenging mitochondrial superoxide [24]. Reduced MnSOD levels in DM may raise ROS levels and be a major factor in the pericyte loss of DR. Research has indicated that there is a substantial difference in MnSOD transcription in the DR group compared to the NO-DR group, and that the DR group has increased DNA methylation of the MnSOD promoter [25]. This suggests that in the retina of diabetics, hypermethylation of a specific promoter and decreased MnSOD activity occur, which ultimately lead to the development of DR.

Inflammation

Inflammation is a chronic manifestation of raised blood sugar which activates inflammatory response, increased pro-inflammatory cytokines, acute phase proteins, chemokines causing retinal angiogenesis and neurodegeneration [26]. Early DR alterations in the retina include low level chronic leukocyte activation, recurrent capillary blockage and progression, and retinal ischemia. Activated microglia found in the retinal plexiform layer control retinal inflammation [27]. In rats with DR, it has been demonstrated that suppression of proinflammatory chemicals prevents degenerative processes [28].

In DR, DNA methylation of promoter of TNF gene decreases causing increased expression of TNF which is a proinflammatory cytokine resulting in inflammation [29].

Transforming Growth Factor Beta-1 (*TGFβ1*), Monocyte Chemoattractant Protein-1 (*MCP-1*), and Tumour Necrosis Factor Superfamily Member 2 (*TNFSF2*), which together play a role in the development of vascular complications in DM [30-32]. DNA methylation in the *TGFβ1*, *MCP-1* and *TNFSF2* genes are also significantly reduced in DR patients. These findings prove that hypomethylation in the promoter of these inflammatory response-related genes participates in DR development.

An important intergenic Long Non-Coding RNA (lncRNA) known for its influence on cancer's epigenetic mechanisms is Metastatic-Associated Lung Adenocarcinoma Transcript 1 (*MALAT1*) [33]. *MALAT1* may control the inflammatory response via independent and dependent mechanisms, which collectively help to create a complicated inflammatory response that leads to the development of DR [34].

Neovascularization

Capillary non-perfusion, retinal ischemia stimulates pro-angiogenic factors in the PDR stage of DR [35]. The stimulation of intricate cascade signals in DM patients promotes aberrant neovascularization by elevating angiogenic factors and mediating retinal endothelial cell proliferation [36]. A crucial compensatory mechanism for retinal ischemia is angiogenesis induced by VEGF, which stimulates the proliferation of retinal endothelial cells, increases the amount of VEGF released by both endothelium and non-endothelial cells, and promotes abnormal neovascularization [37].

Increased production of VEGF due to hypomethylation of the VEGF promoter suggests that DNA methylation has a role in the neovascularization and development of PDR processes [38]. Reduced transcription of maternally expressed gene 3 (9MEG-30) in DR inhibits the endothelial-mesenchymal transition and VEGF production, hence impeding the development of DR [39-41]. In human retinal microvascular endothelial cells, DNMT promotes DNA methylation of the MEG3 promoter to inhibit MEG3 expression, speeding up proliferation, migration, and neovascularization (Figure 3) [42].

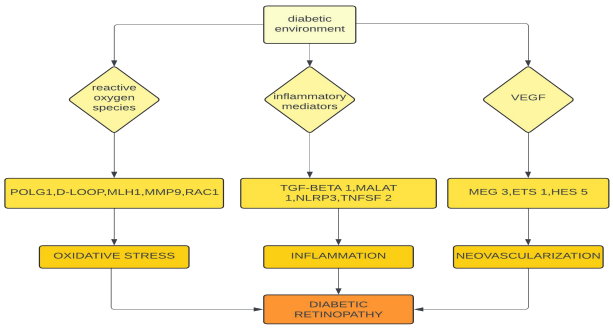

Figure 3. Different genes and process resulting in diabetic retinopathy.

Therapeutic potential of DNA methylation

Diagnosis of DR at a relatively early stage: The clinical signs of diabetic retinopathy occurs long after diagnosis of diabetes mellitus. level of DNA methylation in early stage of the disease can act as a biomarker for early diagnosis of the DR. diabetic patients with retinopathy and those without it have different levels of mtDNA methylation in their peripheral blood [43].

Methylation-specific PCR is a simple and rapid method for quantifying DNA methylation. DNA methylation changes can be non-invasively detected in peripheral blood and can be easily quantifiable using PCR, ELISA, HPLC etc. (Refer Table 1 for different molecular markers in diagnosis of DR).

Table 1. Novel molecular biomarkers related to diabetic retinopathy (SNP: Single Nucleotide Polymorphism).

S. No.	Molecular biomarkers
1	DNA based (SNP's, Telomeres)
2	Epigenetics (DNA methylation, acetylation, histone modification)
3	RNA based (Non-coding RNA including micro-RNA)
4	Proteomics

5	Metabolomics
6	Lipidomics
7	Gllycomics (Analysis of glucose modified lipids or proteins)

Predicting progressive stage of DR (PDR): DNA methylation status may possibly be used as a non-invasive biomarker for predicting the progressive stage of DR, known as PDR. It has been demonstrated that the DNA methylation of some genes in peripheral blood can be used as a prospective biomarker of PDR in patients with type 1 diabetes. These genes include the following: (1) TNF; (2) Chitinase 3-Like Protein 1 (*CHI3L1*), which participates in tissue injury, inflammation, tissue repair, and remodelling responses; (3) Chimerin 2 (*CHN2*), which may be a key element of proximal insulin signalling, playing a role in insulin resistance and growth deficiency; and (4) Gastric Inhibitory Polypeptide Receptor (*GIPR*), which encodes glucose-dependent insulinotropic polypeptide receptor [44-46]. Additionally, PDR patients had lower levels of DNA methylation in these genes [47]. Furthermore, it has been noted that PDR patients have hypomethylated versions of a few particular genes that are implicated in the cytotoxicity pathway mediated by natural killer cells [48]. indicating even further that various methylation patterns may function as encouraging indicators of PDR [49-54].

Predicting effectiveness of drug therapy: The potential utility of methylation status as a predictor of VEGF receptor medication efficacy in cancer cells has been established by the analysis of promoter methylation [55]. At present, however, this type of study in DR is insufficient; nonetheless, additional research on the key genes is anticipated to enable the use of DNAme-related markers to predict the efficacy of DR therapy.

Treatment: Targeting DNA methylation: Risk factors DR include lifestyle choices, environmental variables, dyslipidemia, hyperglycemia, obesity, and Hyperhomocysteinemia (HHcy). These factors might change the epigenetic status of many target organs either separately or in combination [56].

Hyperglycemia: Due to the fact that hyperglycemia is the precursor of aberrant DNA methylation in diabetes, glycaemic management is a crucial preventive intervention in diminishing the likelihood of diabetic retinitis and vision impairment [57-59]. First, when mtDNA and nDNA methylation and Dnmts and Tets activation are unaltered throughout the early stages of diabetes mellitus, the preservation of retinal mitochondria is facilitated by the restoration of appropriate glycaemic management. This demonstrates that the DNA methylation mechanism does not harm the retina of diabetes individuals when blood glucose is controlled early and kept at healthy levels. Furthermore, long-term tight glycaemic management can improve aberrant methylation status and ultimately postpone or stop the development of DR, even in patients who are unable to achieve strict glycaemic control during the first phase of diabetes mellitus [60].

Hyperhomocysteinemia (HHcy): HHcy is a significant contributor to retinal microvascular damage and an independent risk factor for the onset of retinopathy [61]. Furthermore, it might have negative synergistic effects with hyperglycemia [62]. In order to preserve normal DNA methylation status under physiological settings, Hcy can generate SAM and boost Dnmts activity. But in DR, elevated levels of circulating Hcy cause a worldwide rise in retinal DNA methylation [63]. Additional impacts on the methylation of genes essential for mtDNA synthesis result in a harmful cycle of reactive oxygen species and mitochondrial malfunction, which exacerbates the development of DR [64]. Thus, in order to prevent DR, we can implement a variety of strategies that regulate the amount of Hcy in the air. For instance, deficiencies in folic acid and vitamin B_{12} may contribute to Hypochloremia (HCy). These nutrients are necessary for the correct metabolism of Hcy [65]. Thus, using folic acid and vitamin B_{12} to treat pre-existing HHcy may help lower the likelihood of developing DR [66].

Hyperlipidaemia/obesity: Some cross-sectional and longitudinal studies indicate an association between an increased risk of DR and hyperlipidemia/obesity [67]. In DM rats, hyperlipidemia exaggerates hyperglycemia-induced DNA methylation, increases Dnmts and Tet2, and alters methylation of mtDNA and RAC1. Consequently, mitochondrial damage and retinopathy are accelerated [68].

Dnmt inhibitors: The Dnmt inhibitors 5-azacytidine and 5-aza-20-deoxycytidine have been granted approval by the US Food and Drug Administration for the treatment of myeloid malignancies and cutaneous T-cell lymphoma [69]. Research on Dnmt inhibitors has evolved from non-selective to selective. first-generation Dnmt inhibitors including 5-azacitidine and 5-aza-2'-deoxycytidine inhibit all three DNMTS (1, 3a, and 3b); however, subsequent research revealed that nanaomycin A and GSK3685032 specifically inhibited Dnmt3b and Dnmt1, respectively [70].

Glycaemic management alone cannot instantly improve the DNA methylation-hydroxy methylation pathway in the retina. Direct Dnmt inhibition, on the other hand, may enhance aberrant DNA methylation during this reversal phase, so mitigating persistent mitochondrial dysfunction and delaying or preventing the onset of DR. For example, during times of good glycaemic control after recovery of hyperglycemia, direct suppression of Dnmts can restore DNA methylation and gene transcription of the *MLH1* gene. Furthermore, throughout the development of DR, one mechanism that may compromise antioxidant defence systems is DNA methylation. If Dnmt inhibitors are administered prior to the acceleration of apoptosis in retinal capillary cells, they can prevent mtDNA damage by inhibiting further DNA methylation of genes crucial for preserving mitochondrial homeostasis. 5-aza-2'-deoxycytidine, a DNA-demethylating medication, has the potential to treat DR since it can reverse elevated Dnmts and control the expression of antioxidant enzymes to increase antioxidative capability [71]. Despite its non-selective nature and its ability to block three different Dnmts, 5-aza-2'-deoxycytidine has not been observed to cause considerable damage to retinal structure [72]. This finding may be connected to the fact that all Dnmts are more active in a diabetic environment. To increase efficacy and lessen negative effects, selective inhibitors can be studied more as there hasn't been much study done in this field. Dnmt1, for instance, exhibits unique and tissue-specific features [73]. It will be possible to investigate the viability of using a Dnmt1 selective inhibitor to treat DR by learning more about the distribution properties of Dnmt1 in retinal tissue.

Folic acid and vitamin B_{12}: For DNA methylation, Folic Acid (FA) is a dietary methyl donor, and vitamin B_{12} is a cofactor in the process of moving the methyl group from FA to DNA [74,75]. Although studies indicate that low FA levels can cause both hypomethylation and hypermethylation, misregulating DNA methylation, FA has been utilised extensively in the clinic to treat DR [76]. A blood folic acid/ vitamin B_{12} deficit is linked to a higher risk of developing diabetes, and taking these nutrients supplements lowers the risk of developing diabetes. Further supporting the importance of FA and vitamin B_{12} in DR treatment, implies that deficits in these nutrients may influence the equilibrium of DNA methylation and so facilitate the development of DR. Nevertheless, more research is required to fully understand the DNA methylation-related mechanism behind its therapeutic action.

Synergistic therapy with current therapies: We think that Dnmts inhibitors can be used in addition to existing medicines, based on the results of the present research. The restrictions and side effects of traditional therapy can be reduced with this synergistic therapy, and the therapeutic outcome for DR can be enhanced.

Anti-VEGF therapy is mainly used in PDR patients, and has a significant effect on neovascularization and diabetic macular edema, which can improve visual acuity [77]. The main problems are as follows: A large part of the population is not sensitive to anti-VEGF therapy [78]. Studies have demonstrated that the therapeutic efficacy of the

- A large part of the population is not sensitive to anti-VEGF therapy [78]. Studies have demonstrated that the therapeutic efficacy of the VEGF-targeted medicines can be influenced by the DNA methylation status of the VEGF receptor promoter. Therefore, the DR patients who are not sensitive to anti-VEGF therapy may be associated with the abnormal DNA methylation of VEGF receptor promoter caused by diabetes. Anti-VEGF therapy in combination with Dnmt inhibitors may be a potential approach to improve the therapeutic effects of those special DR patients.
- Anti-VEGF therapy is mainly used in the PDR stage with obvious ocular symptoms, and Dnmt inhibitors are expected to prevent entering the PDR stage. As mentioned previously, DNA methylation in peripheral blood is expected as a diagnostic biomarkers of early DR. With close follow-up before the diagnosis of PDR, Dnmt inhibitors can be used to reverse epigenetic abnormalities caused by hyperglycemia in a short period of time. In conjunction with effective blood glucose management in the later stages, it is extremely encouraging to postpone or even avert the progression of DR to PDR.
- Considering the chronic course of DR and the short-term efficacy of anti-VEGF therapy, PDR patients should be regularly and frequently injected with anti-VEGF for a period of time. If this treatment is interrupted, it could be disastrous and result in irreparable eyesight loss [79]. However, many patients find it challenging to attain adequate compliance due to the inconvenience of follow-up and the significant financial load. Dnmts inhibitors can be utilised as adjunctive therapy to increase patient compliance by extending the duration of effect and lowering associated costs.

Discussion

In a nutshell, methylation studies on whole genome and specific genes are very limited on diabetic retinopathy. Maghbooli Zhila, et al. [80] in a case control study done on 168 patients using high pressure liquid chromatography concluded that whole DNA methylation levels are significantly higher in DR patients. Zhuo Chen, et al. compared DNA methylation profile in genomic DNA of whole blood from 32 cases with 31 controls without retinopathy [81]. 153 loci depicted hypomethylation and 225 depicted hypermethylation and also concluded DNA methylation persist at certain loci associated with glycemia for several years supporting and epigenetic basis for metabolic memory.

Shikha Tewari, et al. conducted an animal study to understand the mechanism of DNA methylation status of *POLG1* promoter in streptozocin induced diabetic retinopathy rat models [82]. Isolated retinal endothelial cells exposed to high glucose compared to those exposed to normal glucose showed continued POLG1 downregulation and CpG island hypermethylation, which continued to remain same even after three months of glycemia control following poor glycemic control supporting metabolic memory. Manisha Mishra and Renu Kowluru conducted yet another animal study to investigate possible crosstalk between mtDNA methylation and base mismatch in the development of diabetic retinopathy [83]. The effect of inhibition of DNMT by 5 aza-2'-deoxycytidine on glucose induced mt-DNA base mismatch was investigated in human endothelial cell. An *in vivo* model of retinal microvasculature from diabetic mice overexpressing sod2 was used to study effect of deamination on increased base mismatch. Inhibition of DNA methylation, regulation of cytosine deamination, significantly inhibited an increase in base mismatch and prevented mitochondrial dysfunction. Overexpression of sod2 in mice also prevented diabetes induced D-loop hypermethylation and increase in base mismatch. Inhibition of DNA methylation limits the availability of methylated cytosine for deamination suggesting cross talk between DNA methylation and base mismatch. The crosstalk continued even after termination of hyperglycemia, suggesting its role in metabolic memory. Ying Zhu, et al. concluded DNMT 1 mediated PPAR-alpha methylation promotes apoptosis and increase ROS level of human retinal cell pericytes obtained from experimental animal model and causes damage of retinal tissue in DR mice [84].

Similar studies done by Yaki Li, et al. for THBS1, He Yui, et al. for *MEG3*, etc. suggested a close relation of DNA methylation and Diabetic retinopathy [85].

Conclusion

In summary, oxidative stress, inflammation, neovascularization, and metabolic memory are only a few of the intricate pathophysiological processes whereby DNA methylation plays a crucial role in the onset and progression of DR. It may function as a biomarker for DR early detection as well as a possible target for treatment and prevention. However, treatments for DR are still somewhat new and mostly target the neovascularization of PDR patients, while research on DNA methylation is still in its early stages. In the future, DNMTT inhibitors may be used as therapeutics to treat DR. It will be crucial to determine which kind of Dnmt inhibitor controls the antioxidant genes and the precise hypermethylation CpG site of the genes, though, as the majority of these inhibitors that are presently being researched are nonselective. Are there any negative effects, such as myelosuppression and neurological damage, that are linked to cancer treatment when these inhibitors are used to treat DR? Do these inhibitors profoundly alter the retina's morphology and functionality? Do people with diabetes grow tolerant to sudden suppression of retinal Dnmts? Is it possible for DNMTS inhibitors to compensate for the lack of anti-VEGF medications and cortisol implants?Future investigations must take into account each of these queries. Therefore, it is anticipated that additional study on DNA methylation will lead to new discoveries in the therapy of DR and provide people who are blind from DR with hope again.

Authors Contributions and Authors Information

All authors searched for the literature and wrote and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest Funding

None.

Funding

Not applicable.

References

1. Moore, L.D., et al. "DNA methylation and its basic function." *Neuropsychopharmacology* 38.1 (2013): 23-38.
2. Sergeeva, A., et al. "Mechanisms of human DNA methylation, alteration of methylation patterns in physiological processes and oncology." *Gene* 875 (2023): 147487.
3. Pappalardo, X.G., & Barra, V. "Losing DNA methylation at repetitive elements and breaking bad." *Epigenetics Chromatin* 14.1 (2021): 25.
4. Zhang, W., & Xu, J. "DNA methyltransferases and their roles in tumorigenesis." *Biomark Res* 5 (2017): 1-8.
5. Jin, B., et al. "DNA methylation: Superior or subordinate in the epigenetic hierarchy?." *Genes Cancer* 2.6 (2011): 607-617.
6. Close, P., et al. "Dynamic regulation of tRNA modifications in cancer." *Cancer Noncod RNAs* (2018): 163-186.
7. Okano, M., et al. "DNA methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development." *Cell* 99.3 (1999): 247-257.
8. Cheng, X., & Blumenthal, R.M. "Mediating and maintaining methylation while minimizing mutation: Recent advances on mammalian DNA methyltransferases." *Curr Opin Struct Biol* 75 (2022): 102433.
9. Eriksson, A., et al. "Epigenetic aberrations in acute myeloid leukemia: Early key events during leukemogenesis." *Exp Hematol* 43.8 (2015): 609-624.
10. Yin, Y., et al. "Impact of cytosine methylation on DNA binding specificities of human transcription factors." *Science* 356.6337 (2017): eaaj2239.
11. Cai, C., et al. "DNA methylation in diabetic retinopathy: Pathogenetic role and potential therapeutic targets." *Cell Biosci* 12 (2022): 186.
12. Zhang, Q., et al. "Differential regulation of the Ten-Eleven Translocation (TET) family of dioxygenases by O-linked β-N-acetylglucosamine Transferase (OGT)." *J Biol Chem* 289.9 (2014): 5986-5996.
13. Phillips, T., & Shaw, K. "Chromatin remodeling in eukaryotes." *Nat Educ* 1.1 (2008): 209.
14. Wu, J., et al. "DNA Methylation plays important roles in retinal development and diseases." *Exp Eye Res* 211 (2021): 108733.
15. Dvoriantchikova, G., et al. "The potential role of epigenetic mechanisms in the development of retinitis pigmentosa and related photoreceptor dystrophies." *Front Genet* 13 (2022): 827274.
16. Pizzino, G., et al. "Oxidative stress: Harms and benefits for human health." *Oxid Med Cell Longev* 2017.1 (2017): 8416763.
17. Kowluru, R.A., & Shan, Y. "Role of oxidative stress in epigenetic modification of *MMP*-9 promoter in the development of diabetic retinopathy." *Graefes Arch Clin Exp Ophthalmol* 255.5 (2017): 955-962.
18. Kang, Q., & Yang, C. "Oxidative stress and diabetic retinopathy: Molecular mechanisms, pathogenetic role and therapeutic implications." *Redox Biol* 37 (2020): 101799.
19. Zorov, D.B., et al. "Mitochondrial Reactive Oxygen Species (ROS) and ROS-induced ROS release." *Physiol Rev* 94.3 (2014): 909-950.
20. Facchinello, N., et al. "Efficient clofilium tosylate-mediated rescue of POLG-related disease phenotypes in zebrafish." *Cell Death Dis* 12.1 (2021): 100.
21. Rashid, S., et al. "MLH1 deficiency leads to deregulated mitochondrial metabolism." *Cell Death Dis* 10.11 (2019): 795.
22. Mohammad, G., et al. "Functional regulation of an oxidative stress mediator, Rac1, in diabetic retinopathy." *Mol Neurobiol* 56 (2019): 8643-8655.
23. Ramakrishnan, S.K., et al. "PPARα (peroxisome proliferator-activated receptor α) activation reduces hepatic CEACAM1 protein expression to regulate fatty acid oxidation during fasting-refeeding transition." *J Biol Chem* 291.15 (2016): 8121-8129.
24. Li, L., & Yang, X. "The essential element manganese, oxidative stress, and metabolic diseases: Links and interactions." *Oxid Med Cell Longev* 2018.1 (2018): 7580707.
25. Duraisamy, A.J., et al. "Epigenetic modifications in peripheral blood as potential noninvasive biomarker of diabetic retinopathy." *Transl Vis Sci Technol* 8.6 (2019): 43-43.
26. Chen, H., et al. "Identification of NLRP3 inflammation-related gene promoter hypomethylation in diabetic retinopathy." *Invest Ophthalmol Vis Sci* 61.13 (2020): 12-12.
27. Kinuthia, U.M., et al. "Microglia and inflammatory responses in diabetic retinopathy." *Front Immunol* 11 (2020): 564077.
28. Vallejo, S., et al. "The interleukin-1 receptor antagonist anakinra improves endothelial dysfunction in streptozotocin-induced diabetic rats." *Cardiovasc Diabetol* 13 (2014): 1-13.
29. Agardh, E., et al. "Genome-wide analysis of DNA methylation in subjects with type 1 diabetes identifies epigenetic modifications associated with proliferative diabetic retinopathy." *BMC Med* 13 (2015): 1-9.
30. Saucedo, L., et al. "Ocular TGF-β, matrix metalloproteinases, and TIMP-1 increase with the development and progression of diabetic retinopathy in type 2 diabetes mellitus." *Mediators Inflamm* 2021 (2021): 9811361.
31. Stepp, M.A, & Menko, A.S. "Immune responses to injury and their links to eye disease." *Transl Res* 236 (2021): 52-71.
32. Rosa, J.S., et al. "*Ex vivo* TCR-induced leukocyte gene expression of inflammatory mediators is increased in type 1 diabetic patients but not in overweight children." *Diabetes Metab Res Rev* 26.1 (2010): 33-39.
33. Jiao, F., et al. "Long noncoding RNA MALAT-1 enhances stem cell-like phenotypes in pancreatic cancer cells." *Int J Mol Sci* 16.4 (2015): 6677-6693.
34. Biswas, S., et al. "MALAT1: An epigenetic regulator of inflammation in diabetic retinopathy." *Sci Rep* 8.1 (2018): 6526.
35. Biswas, S., et al. "The long non-coding RNA HOTAIR is a critical epigenetic mediator of angiogenesis in diabetic retinopathy." *Invest Ophthalmol* Vis Sci 62.3 (2021): 20-20.
36. Nentwich, M.M., & Ulbig, M.W. "Diabetic retinopathy-ocular complications of diabetes mellitus." *World J Diabetes* 6.3 (2015): 489.
37. Yang, Y., et al. "MicroRNA-15b targets VEGF and inhibits angiogenesis in proliferative diabetic retinopathy." *J Clin Endocrinol Metab* 105.11 (2020): 3404-3415.

38. Sundrani, D.P., et al. "Differential placental methylation and expression of VEGF, FLT-1 and KDR genes in human term and preterm preeclampsia." *Clin Epigenetics* 5 (2013): 6.

39. Zhang, D., et al. "LncRNA MEG3 overexpression inhibits the development of diabetic retinopathy by regulating TGF-β1 and VEGF." *Exp Ther Med* 16.3 (2018): 2337-2342.

40. Di, Y., et al. "Maternally expressed gene 3 regulates retinal neovascularization in retinopathy of prematurity." *Neural Regen Res* 17.6 (2022): 1364-1368.

41. He, Y., et al. "DNMT1-mediated lncRNA MEG3 methylation accelerates endothelial-mesenchymal transition in diabetic retinopathy through the PI3K/Akt/mTOR signaling pathway." *Am J Physiol Endocrinol Metab* 320.3 (2021): E598-E608.

42. Chen, J., et al. "Long non-coding RNA MEG3 inhibits neovascularization in diabetic retinopathy by regulating microRNA miR-6720-5p and cytochrome B5 reductase 2." *Bioengineered* 12.2 (2021): 11872-11884.

43. Conte, C., et al. "Metabolic memory in diabetes: Permanent scar, legacy, or ongoing domino effect?." *Cardiovasc Res* 118.1 (2022): 4-6.

44. Suliman, S.G., et al. "Severe insulin resistance and intrauterine growth deficiency associated with haploinsufficiency for *INSR* and *CHN2*: New insights into synergistic pathways involved in growth and metabolism." *Diabetes* 58.12 (2009): 2954-2961.

45. Ast, J., et al. "Reagents and models for detecting endogenous GLP1R and GIPR." *EBioMedicine* 74 (2021).

46. Kim, J., et al. "Promoter methylation status of VEGF receptor genes: A possible epigenetic biomarker to anticipate the efficacy of intracellular-acting VEGF-targeted drugs in cancer cells." *Epigene*tics 7.2 (2012): 191-200.

47. Ducos, C., et al. "Diabetic retinopathy in well-controlled type 2 diabetes: Role of glycaemic memory." *Diabetes Metab* 47.1 (2021): 101156.

48. Lee, C., et al. "Hyperglycemic memory in metabolism and cancer." *Horm Mol Biol Clin Investig* 26.2 (2016): 77-85.

49. Villeneuve, L.M., & Natarajan, R. "The role of epigenetics in the pathology of diabetic complications." *Am J Physiol Renal Physiol* 299.1 (2010): F14-F25.

50. Kowluru, R.A. "Diabetic retinopathy, metabolic memory and epigenetic modifications." *Vision Res* 139 (2017): 30-38.

51. Reddy, M.A., et al. "Epigenetic mechanisms in diabetic complications and metabolic memory." *Diabetologia* 58 (2015): 443-455.

52. Wang, Z., et al. "Metabolic memory in mitochondrial oxidative damage triggers diabetic retinopathy." *BMC Ophthalmol* 18 (2018): 258.

53. Natarajan, R. "Epigenetic mechanisms in diabetic vascular complications and metabolic memory: The 2020 Edwin Bierman Award Lecture." *Diabetes* 70.2 (2021): 328-337.

54. Chen, Z., et al. "DNA methylation mediates development of HbA1c-associated complications in type 1 diabetes." *Nat Metab* 2.8 (2020): 744-762.

55. Cai, W.J., et al. "Changes of DNA methylation pattern in metabolic pathways induced by high-carbohydrate diet contribute to hyperglycemia and fat deposition in grass carp (*Ctenopharyngodon idellus*)." *Front Endocrinol* 11 (2020): 398.

56. Zhu, W., et al. "Association of obesity and risk of diabetic retinopathy in diabetes patients: A meta-analysis of prospective cohort studies." *Medicine* 97.32 (2018): e11807.

57. Xie, M.Y., et al. "5-aza-2'-deoxycytidine in the regulation of antioxidant enzymes in retinal endothelial cells and rat diabetic retina." *Int J Ophthalmol* 12.1 (2019): 1.

58. Wat, N., et al. "Associations between diabetic retinopathy and systemic risk factors." *Hong Kong Med J* 22.6 (2016): 589.

59. Fotiou, P., et al. "Vitamin status as a determinant of serum homocysteine concentration in type 2 diabetic retinopathy." *J Diabetes Res* 2014.1 (2014): 807209.

60. Suzuki, M.M., & Bird, A. "DNA methylation landscapes: Provocative insights from epigenomics." *Nat Rev Genet* 9.6 (2008): 465-476.

61. Shi, C., et al. "Nutritional and medical food therapies for diabetic retinopathy." *Eye Vis* 7 (2020): 1-16.

62. Mohammad, G., & Kowluru, R.A. "Homocysteine disrupts balance between MMP-9 and its tissue inhibitor in diabetic retinopathy: The role of DNA methylation." *Int J Mol Sci* 21.5 (2020): 1771.

63. Elmasry, K., et al. "Epigenetic modifications in hyperhomocysteinemia: Potential role in diabetic retinopathy and age-related macular degeneration." *Oncotarget* 9.16 (2018): 12562.

64. Kowluru, R.A. "Diabetic retinopathy: Mitochondria caught in a muddle of homocysteine." *J Clin Med* 9.9 (2020): 3019.

65. Fu, Y., et al. "Hyperhomocysteinaemia and vascular injury: Advances in mechanisms and drug targets." *Br J Pharmacol* 175.8 (2018): 1173-1189.

66. Liew, S.C., & Gupta, E.D. "Methylenetetrahydrofolate Reductase (MTHFR) C677T polymorphism: Epidemiology, metabolism and the associated diseases." *Eur J Med Genet* 58.1 (2015): 1-10.

67. Yau, J.W.Y., et al. "Global prevalence and major risk factors of diabetic retinopathy." *Diabetes Care* 35.3 (2012): 556-564.

68. Kowluru, R.A. "Retinopathy in a diet-induced type 2 diabetic rat model and role of epigenetic modifications." *Diabetes* 69.4 (2020): 689-698.

69. Jin, B., et al. "DNA methyltransferase 3B (DNMT3B) mutations in ICF syndrome lead to altered epigenetic modifications and aberrant expression of genes regulating development, neurogenesis and immune function." *Hum Mol Genet* 17.5 (2008): 690-709.

70. Medina-Franco, J.L., et al. "7-Aminoalkoxy-quinazolines from epigenetic focused libraries are potent and selective inhibitors of DNA methyltransferase 1." *Molecules* 27.9 (2022): 2892.

71. Xie, M., et al. "Effects of 5-aza-2'-deoxycytidine and trichostatin A on high glucose-and interleukin-1β-induced secretory mediators from human retinal endothelial cells and retinal pigment epithelial cells." *Mol Vis* 20 (2014): 1411.

72. Kowluru, R.A., & Mohammad, G. "Epigenetics and mitochondrial stability in the metabolic memory phenomenon associated with continued progression of diabetic retinopathy." *Sci Rep* 10.1 (2020): 6655.

73. Majumdar, S., et al. "Aberrant DNA methylation and prostate cancer." *Curr Genomics* 12.7 (2011): 486-505.

74. Bestry, M., et al. "Association of prenatal alcohol exposure with offspring DNA methylation in mammals: A systematic

review of the evidence." *Clin Epigenetics* 14 (2022): 12.

75. Pennington, K.L., & DeAngeli, M.M. "Epigenetic mechanisms of the aging human retina." *J Exp Neurosci* 9 (2016): 51-79.
76. Crider, K.S., et al. "Folate and DNA methylation: A review of molecular mechanisms and the evidence for folate's role." *Adv Nutr* 3.1 (2012): 21-38.
77. Wong, T.Y., et al. "Erratum: Diabetic retinopathy." *Nat Rev Dis Primers* 2.1 (2016).
78. Chatziralli, I., & Loewenstein, A. "Intravitreal anti-vascular endothelial growth factor agents for the treatment of diabetic retinopathy: A review of the literature." *Pharmaceutics* 13.8 (2021): 1137.
79. Young, B.K., et al. "A caveat about financial incentives for anti-vascular endothelial growth factor therapy for diabetic retinopathy." *Am J Ophthalmol* 243 (2022): 77-82.
80. Maghbooli, Z., et al. "Global DNA methylation as a possible biomarker for diabetic retinopathy." *Diabetes Metab Res Rev* 31.2 (2015): 183-189.
81. Chen, Z., et al. "Epigenomic profiling reveals an association between persistence of DNA methylation and metabolic memory in the DCCT/EDIC type 1 diabetes cohort." *Proc Natl Acad Sci* 113.21 (2016): E3002-E3011.
82. Tewari, S., et al. "Mitochondria DNA replication and DNA methylation in the metabolic memory associated with continued progression of diabetic retinopathy." *Invest Ophthalmol Vis Sci* 53.8 (2012): 4881-4888.
83. Mishra, M., & Kowluru, R.A. "The role of DNA methylation in the metabolic memory phenomenon associated with the continued progression of diabetic retinopathy." *Invest Ophthalmol Vis Sci* 57.13 (2016): 5748-5757.
84. Zhu, Y., et al. "DNMT1-mediated PPARα methylation aggravates damage of retinal tissues in diabetic retinopathy mice." *Biol Res* 54 (2021).
85. Li, Y., et al. "Genetic regulation of THBS1 methylation in diabetic retinopathy." *Front Endocrinol* 13 (2022): 991803.

Cite this article: Shaista, et al. "Epigenetic Insights: DNA Methylation and Gene Expression in Diabetic Retinopathy–A Mini Review". J Diabetes Metab, 2024, 15(11), 1-8.