Genes & Diseases (2021) 8, 279–286

Available online at www.sciencedirect.com

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REVIEW ARTICLE

Does epigenetics have a role in age related macular degeneration and diabetic retinopathy?

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Received 25 September 2019; accepted 6 January 2020
Available online 23 January 2020

KEYWORDS
Age-related macular degeneration (AMD);
Diabetic Retinopathy (DR);
Epigenetics;
Gene expression

Abstract Epigenetic mechanisms play an important part in the regulation of gene expression and these alterations may induce long-term changes in gene function and metabolism. They have received extensive attention in bridging the gap between environmental exposures and disease development via their influence on gene expression. DNA methylation is the earliest discovered epigenetic alteration. In this review, we try to examine the role of DNA methylation and histone modification in Age related macular degeneration (AMD) and Diabetic Retinopathy (DR), its vascular complications and recent progress. Given the complex nature of AMD and DR, it is crucial to improve therapeutics which will greatly enhance the quality of life and reduce the burden for millions of patients living with these potentially blinding conditions.

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URL: http://cdn.bu.ac.in/faculty_data/hgmb_dr_vb.pdf.
Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2020.01.003

Introduction

Epigenetic modifications are changes in gene expression and phenotype, without alterations in the DNA sequence.[1] Of late, epigenetic modifications have been emphasized as chief mechanisms for the advancement of several diseases.[2] Epigenetic modifications include DNA methylation, histone modification and non-coding RNAs[3,4] and they activate multiple signaling pathways and regulate gene expression in eyes.[5] Exploring the interactions between epigenome, genome, and environmental changes will increase the understanding of epigenetic-disease mechanisms.[6] DNA methylation is the earliest discovered and most important epigenetic modification, which is exerted by DNMTs at the 5-position of cytosine residues in CpG dinucleotides,[7] which are often grouped in clusters, as CpG islands. DNA methylation of promoter CpG islands can control gene expression[8] and hence alterations in these processes may induce long-term changes in gene function and metabolism.[9] Conventionally, epigenetic changes were static in gene expression regulation, but, this impression is now being reformed and epigenetic marks, including DNA methylation are dynamic. Though the studies are limited, its known that, epigenetic modifications are protective and it can be reversible, this can be used as therapeutic tools targeting vascular complications. In this review, we examined the role of DNA methylation and histone modification in AMD and DR, its vascular complications and recent progress that have significantly accelerated this field.

Environment gene interactions in AMD

Advances in ageing affects molecular, "cellular and physiological levels" which results in AMD.[10] About 8.7% of all cases of blindness globally are contributed by AMD and it is the primary cause of visual loss in technologically advanced countries.[11] It has been assessed that about 18.9 million will be affected by the year 2040.[12] At present, treatments exist only for the exudative or 'wet' form of AMD and these rely on angiogenesis inhibitors.[13] There is no treatment for atrophic 'dry' AMD, characterized by RPE loss and progressive neuroretinal cellular dysfunction. Extensive studies across the world have highlighted the involvement of genomics to the risk of developing AMD.[14]

Drusen formation leads to visual disturbances characterized by the role of epigenetics in AMD pathogenesis and the epigenetic mechanisms show a promising gap between environmental exposure and disease development, their influence on gene expression.[15] Such studies will allow researchers to generate much-needed therapeutics in preventing disease progression and therapeutic improvement, will greatly advance the value of life and cut the disease burden for millions of patients with blinding conditions.[16]

Soft drusen formation between Bruch's membrane and retinal pigmented epithelial (RPE) and loss of RPE cells in the macular region will eventually leads to permanent vision loss.[17] Hence, it is crucial to appreciate that cellular phenotype and function are not exclusively decided by genetic information. The non-genetic factors interact with epigenome and alter the expression of gene.[18] Therefore all these three factors viz; environment, gene and epigenetics are involved in the AMD pathogenesis. Environmental risk factors, including diet, obesity, smoking, sun exposure, and age, may elicit epigenetic changes that accumulate over a lifetime, eventually resulting in altered gene expression. In AMD, ageing is an important factor for gene silencing due to epigenetic alterations.[19] At a molecular level, epigenetic modifications affect protein, metabolic and transcriptomic activities in diseases and can be used as targets for new drugs. These epigenetic modifications are represented in Table 1 and the changes that occur in retina of AMD patients are illustrated in Fig. 1.

DNA methylation status in AMD

Both genetic and environmental elements contribute to the risk of AMD, it is less clear how these 2 systems interact. One type of epigenetic mark that has been explored in a number of studies is the difference in DNA methylation patterns between cases and controls. Currently, only inadequate studies have been reported in ocular diseases related to epigenetics which include age-related macular degeneration (AMD), diabetic retinopathy, cataract, pterygium, retinoblastoma, uveal melanoma, glaucoma, keratitis, and uveitis.[20–22] In this context, DNMTs activity was found to be higher in AMD patients than in controls. AMD patients also exhibited up-regulation of DNMT1 and DNMT3B expression. It has been observed that expression of HIF1a is downregulated by HDAC1 and expression of VEGF is downregulated by HDAC7 and promotes the angiogenesis genes.[23,24] Epigenetic changes involved in inflammatory cytokines and T cells leads to another hallmark of AMD[25] and Interleukin (IL)-17 receptor C promoter demethylation enhances inflammatory response.[26] A study on methylation status revealed an elevated level of mRNA expression in response to IL17RC promoter hypomethylation in retina and peripheral blood of AMD patients and this epigenetic modification may impact the proinflammatory activity of monocytes in AMD pathogenesis, although further research is needed to confirm these results.[12] Wei et al[26] assessed methylation changes at the IL17RC promoter in discordant siblings for AMD as well as in an AMD case–control study and evaluated IL17RC expression in the eyes and blood of AMD patients and showed that hypomethylation of the IL17RC promoter led to an elevated expression of its protein and mRNA, suggesting that the DNA methylation pattern and expression of IL17RC may potentially serve as a biomarker for the diagnosis of AMD. In contrast, Oliver et al[27] could not find any evidence for differential methylation between AMD and controls, therefore concluding that hypomethylation of the promoter region will not be an applicable biomarker for AMD diagnosis, further pin-pointing the requirement for more studies in this area. Similarly, Oxidative damage induces decreased mRNA and protein levels of detoxification enzymes, such as glutathione S-transferase (GSTM1 and GSTM5) in RPE cells correlated with GSTM1 promoter hypermethylation.[28] In precise, GSTM1 and GSTM5 were subjected to hypermethylation leading to lower amounts of corresponding mRNA and proteins in RPE and choroid which increases the susceptibility to oxidative stress in patients with AMD.[29] Interestingly, both mitochondrial and nuclear

Table 1 Summary of relevant studies on epigenetic modifications in AMD and DR

S.No.	Disease type	Epigenetic Assay	Marker	Results	Ref
1.	DR	DNA methylation	Sirt1 promoter	Hypermethylated *Sirt1* promoter	
2.	DR	Histone acetylation - Quantified using Chromatin Immunoprecipitation (ChIP)	MMP-9 promoter	Overexpression of Sirt1	
3.	Type-I diabetes with proliferative DR	Genome wide analysis (GWAS) and DNA methylation	349 CpG site - 233 unique genes including TNF, CHI3L1 (also known as YKL-40), CHN2, GIPR, GLRA1, GPX1, AHRR, and BCOR	Decreased DNA methylation	60
4.	DR	DNA methylation	MMP-9 promoter	SIRT1 gene and protein expressions were decreased and increased acetylation of NF-κB at MMP-9 promoter and the activity of MMP-9	
5.	DR	Histone modification - Streptozotocin-induced diabetic rats	MMP-9 promoter	decreased H3K9me2 and increased Ac-H3K9 and p65 at the retinal MMP-9 promoter	61
6.	DR	Histone modification - H3k20me3 and H3k9 NF-κB p65	Sod2	Increased H4K20me3, acetyl H3K9, and NF-κB p65 at the promoter and enhancer of retinal sod2	64
7.	DR	DNA methylation	POLG	down-regulated, the D-loop region and Hypermethylation at regulatory region	58
8.	DR	Histone Modification — Chromatin Immunoprecipitation (human retinal endothelial cells)	MMP-9 promoter	elevated H3K27me3 levels	
9.	DR	Histone modification — Chromatin immunoprecipitation	H3K4 methylation in Nrf2 binding at Gclc-ARE4	H3K4me3 and H3K4me1 decreased	45
10.	DR	Histone modification — chromatin-immunoprecipitation and co-immunoprecipitation	MMP-9 promoter	hyperacetylaation	56
11.	DR	Global DNA methylation - 5-methylcytosine content was assessed by reversed-phase high-pressure liquid chromatography of peripheral blood leukocytes	Global DNA methylation	increasing trend in global DNA methylation levels	61
12.	DR	DNA methylation	D- loop	Methylation in retinal capillary cells	45
13.	DR	DNA methylation	Cyt b region	Increased methylation	45
14.	DR	Histone modification -	Keap1 promoter	Keap1 promoter increased expression	45

(*continued on next page*)

Table 1 (*continued*)

S.No.	Disease type	Epigenetic Assay	Marker	Results	Ref
		Methylation enriched H3k4me1 and activated setD7			
15.	DR	Histone methylation	H3k4 by a lysine specific histone demethylase (LSD-1)	Transcriptional repression	
16.	DR	DNA methylation	POLG	Hypermethylation of CpG sites at the regulatory regions	63
17.	AMD RPE cells	DNA methylation	GSTM1 and GSTM5 promoters	GSTM1 promoter hypermethylation	30
18.	AMD	DNA methylation	IL17RC promoter	Demethylation	27
19.	AMD -RPE cells	Histone modification	SIRT1 induced deacetylation of the p53 protein	Hypomethylation	15,39
20.	DR and AMD [Human retinal endothelial cells (HRECs) and Retinal Pigmented epithelial cells (ARPE-19)]	miRNA analysis - Hcy induced BRB dysfunction	HDAC, DNMT	MiRNA - HDAC and DNMT activation	37
21.	AMD	DNA methylation	IL17RC promoter	Hypomethylation	27
22.	AMD	DNA methylation	IL17RC promoter	Hypermethylation	28,29
23.	AMD	DNA methylation	GSTM1 and GSTM2	Hypermethylation	31
24.	AMD (in retinas and peripheral blood)	DNA methylation	IL17RC	Hypomethylation	12

Table shows the list of evidence of recent studies on Epigenetic modifications (DNA methylation and histone modifications) in Age related macular degeneration (AMD) and Diabetic Retinopathy (DR).

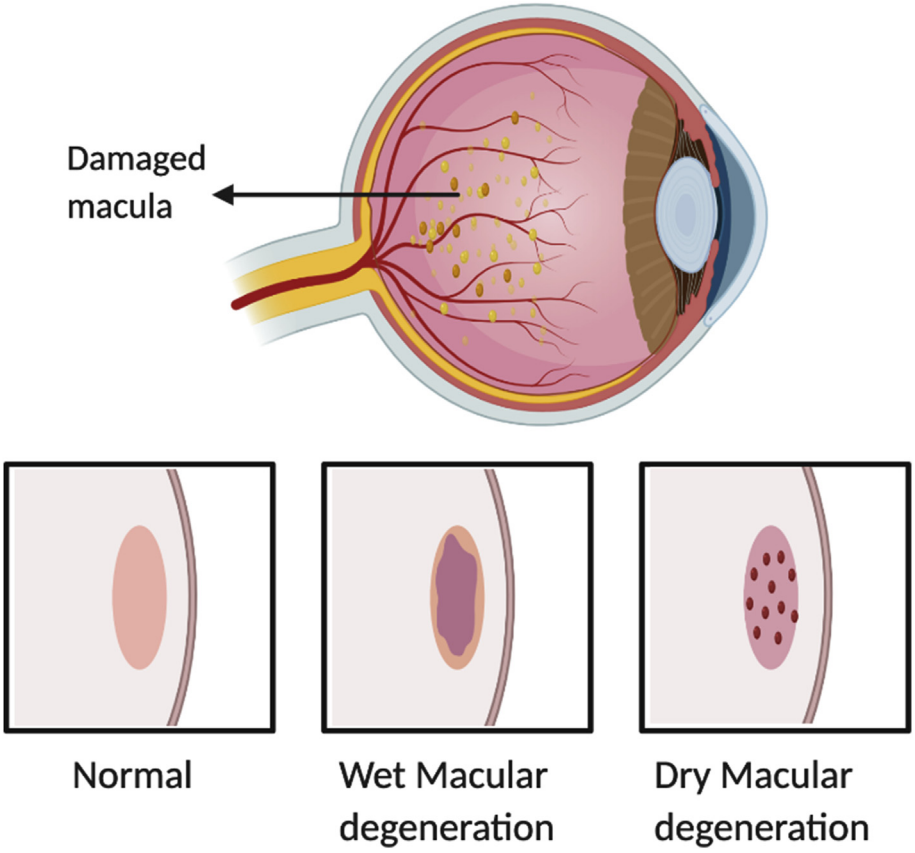

Figure 1 Illustrates the retina structure in normal eye and in the pathogenesis of AMD.

DNA from RPE cells of patients with AMD showed increased oxidative damage, thereby indicating the role of imbalanced redox enzymes in the pathogenesis of AMD.[30]

Histone modifications and chromatin remodeling in AMD

Chromatin is the complex of chromosomal DNA and proteins and DNA is packaged in chromatin around the histone protein units called nucleosomes, which are highly alkaline, and positively charged amine groups[31] which help histone proteins to interact with and bind to the negatively charged phosphate backbone. During acetylation, amines on the histone change into amides; thereby, positive charges on the histone become neutralized and subsequently the capability of the histones to bind with DNA is reduced[32] and this results in expansion of chromatin allowing genetic transcription. The histone deacetylation is catalyzed when histone deacetylases removing acetyl groups and increase the positively charged amine groups of histones and facilitate high-affinity binding between the histones and the phosphate backbone.[33]

Vavilala et al,[34] showed that pure honokiol inhibits the HIF pathway and hypoxia-mediated expression of pro-angiogenic genes in retinal pigmented epithelial (RPE) cell lines using chromatin immunoprecipitation assays, demonstrating that honokiol inhibits binding of HIF to hypoxia-response elements present on VEGF promoter, making it an ideal therapeutic agent for the treatment of ocular neovascular diseases. Subsequently, miRNA pathway analysis has shown their involvement in HDAC and DNMT activation and oxidative stresses, inflammation, hypoxia, and angiogenesis pathways. Hcy-induced epigenetic modifications may be involved in retinopathies associated with HHcy, such as age related macular degeneration and diabetic retinopathy.[35] Previous evidence supports the role of aberrant epigenetic modifications with significant increase in mRNA expression of HDAC1, HDAC3, HDAC6, DNMT1 and DNMT3a in RPE cells of mice with excessive iron levels and, thus, are at a higher risk for AMD.[36] Cultured RPE cells derived from patients with AMD showed hypomethylation of the clusterin promoter as well.[37] Recently, aberrant epigenetic modifications have been identified in the pathogenesis of AMD. This has led to the development of alternative therapies that can alter aberrant chromatin-remodelling processes involved in AMD. These novel therapeutic agents could help to ameliorate the challenges in current treatment. However, epigenetic based treatments are young and more pre-clinical studies are needed to evaluate their mechanism in AMD therapeutics.[38]

Epigenetic changes in diabetic retinopathy (DR)

Diabetic Retinopathy (DR) is a microvascular complication in diabetes and its major initiator is hyperglycemia.[39]

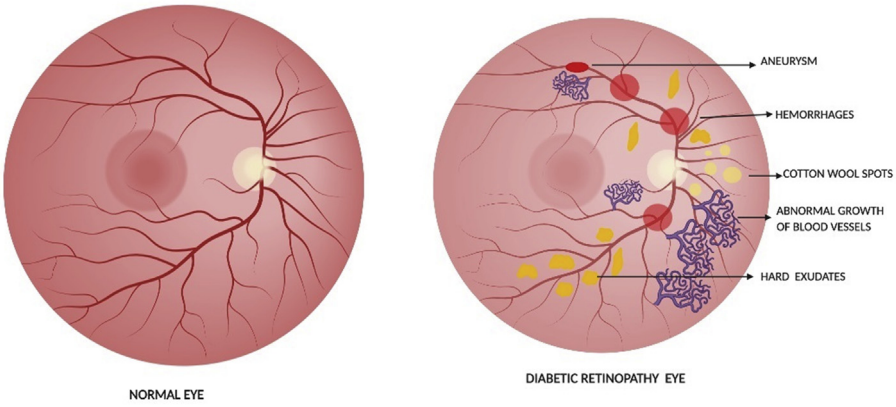

Figure 2 Illustrates the normal retina and in DR pathogenesis.

Development of DR occurred even after hyperglycemia was replaced by normal glycemia.[40–46] Circulation of high glucose is considered as the major instigator for changes in metabolic and deleterious functions.[39,43,47] DNA is highly dynamic and responds to the environmental stimuli by modifying its properties[48] and changes in DNA methylation can lasts for several years.[49] Hyperglycemia initiates metabolic abnormalities which induces genetic alterations in the retina and continues to progress even after hyperglycemia termination.[43,50,51] The epigenetic changes are depicted in Table 1 and the alterations that occur in DR are represented in Fig. 2.

Role of DNA methylation in DR

Reversal of normal glycemia after a period does not reverse the epigenetic changes and mitochondria continues to be dysfunctional.[52,53] In diabetic patients, the DNMT activity is increased,[52] and the maintenance enzyme DNMT1 is upregulated.[54] Tewari et al[52] also demonstrated a significant increase in DNMT activity in the retinal nuclear fraction of the rodents with DR. Differential DNA methylation patterns are observed in the blood samples of patients with proliferative DR as well.[55] Oxidative stress has been known to play a role in DR. The mtDNA hypermethylation plays an important role for the continued imbalance in mitochondrial homeostasis. Diabetes increases Tet activity in the retina and this plays a significant role in the MMP-9 upregulation, an enzyme implicated in mitochondrial damage. Increased binding of Tet2 results in promoter hypomethylation of MMP-9 which results in activation of transcription.[56] Regulation of DNMT1 inhibits glucose-induced mtDNA methylation in retinal endothelial cells, and ameliorates its transcription.[54] Hypermethylation at the regulatory region of DNA polymerase gamma (POLG1) was hypermethylated in DR[57] leads to impairment of the mitochondrial DNA replication system and subsequent apoptosis of retinal capillary cells. Recent studies[58,59] states that diabetic environment favors methylation of CpG dinucleotides forming 5-methylcytosine (5 mC). Continued hypermethylation of the CpG sites at the regulatory region of POLG affects its binding to the mtDNA, compromising the transcriptional activity. Modulation of DNA methylation using pharmaceutic or molecular means could help maintain mitochondria homeostasis, and prevent further progression of DR61. These studies show a strong association between altered methylation in mtDNA and mitochondrial homeostasis.[54,56]

Histone modifications in DR and its therapeutic interventions

Diabetic environment also brings about epigenetic modifications in the retina, and enzymes responsible for modifications of histones and DNA are altered.[43] Hyperglycaemia leads to a significant epigenetic alteration of Sod2 in H4K20me3 and H3K9 in a diabetic rodent model.[61] Reversal of hyperglycemia fails to provide any benefit to epigenetic modifications of Keap1 promoter, suggesting its role in DR.[43] Effect of high glucose on monomethyl H3K4 (H3K4me1), dimethyl H3K4 (H3K4me2), and lysine-specific demethylase-1 (LSD1) was quantified at Sod2 by chromatin immunoprecipitation and histone methylation of retinal Sod2 has an important role in the development of DR and in the metabolic memory phenomenon associated with its continued progression. Similarly, the effect of high glucose on the binding of transcriptional factor Sp1 at Keap1 promoter and histone methylation status was investigated in retinal endothelial cells. Epigenetic modifications at Keap1 promoter by SetD7 facilitate its binding with Sp1, increasing its expression. Polymorphisms in H3K9 and DNMT have also been observed in diabetic vascular complications[62] and the enzyme important for trimethylation of H4K20, is elevated in DR.[63] Epigenetic modifications play an important role in regulation of molecular mechanisms associated with DR and histone modifications in MMP-9. [53] Epigenetic alterations in the MMP-9 promoter region have been identified in human donor eyes with DR[53] and diabetic C57BL/6J mice.[61] Interestingly, experimental evidence has shown that, alterations in histone modifications affects maintenance of mitochondrial homeostasis in DR.[43] Mitochondrial superoxide levels are elevated in the diabetic retina and the dysfunctional mitochondria accelerates retinal cell apoptosis[43,64], which precedes the histopathology in DR, incriminating it in DR. The Sirt1 regulation in diabetes further indicates the role of epigenetic influence on transcriptional suppression.[65] These studies show the

significant link between histone modifications and DR. These kinds of studies are warranted in future to identify a suitable potential biomarker for DR. Analyzing the epigenetic changes and the outcome of the results helps in sensible evaluation of their mechanism of action, specificity and adverse effects. An increased understanding of the complex relationships between genetic, epigenetic, and environmental factors involved in the development of complex diseases, such as degenerative retinal diseases, preventative and therapeutic interventions will reduce the severity and complications arising from such diseases, thereby improving the quality of life for older patients.

Conclusions and future perspectives

As AMD and DR have a complex nature, it is crucial to establish a database of aberrant methylation and acetylation patterns. Eventually, this will allow clinicians to devise individualized therapies and promise patients 'healthy ageing'. DNA methylation plays a critical role in the pathogenesis of retinal disease complications and better understanding of the role and mechanism of DNA methylation and ocular complications can inspire critical implications for the early prevention of these diseases and provide unique opportunities to develop novel therapeutic approaches. However, there are only limited studies related to histone modifications in eye diseases and these kinds of studies are warranted in future. Furthermore, increasing evidence shows that epigenetic modifications are not static, which are dynamic and even reversible and therefore, in this view of epigenetic mechanisms, intervention with pharmaceuticals or other interventions during early course of the disease may ameliorate its complications in later life.

Conflict of Interests

The authors declare no conflict of interests.

Acknowledgements

We thank Vision Research Foundation, Sankara Nethralaya, Chennai for supporting necessary facilities to carry out this review.

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