Title
Further understanding of epigenetic dysfunction of the retinal pigment epithelium in AMD

Journal
Expert Review of Ophthalmology, 15(4)

ISSN
1746-9899

Authors
Nashine, Sonali
Kenney, Maria Cristina

Publication Date
2020-07-03

DOI
10.1080/17469899.2020.1767597

Peer reviewed

HHS Public Access

Author manuscript

Expert Rev Ophthalmol. Author manuscript; available in PMC 2021 March 16.

Published in final edited form as:

Expert Rev Ophthalmol. 2020 ; 15(4): 221–231. doi:10.1080/17469899.2020.1767597.

Further understanding of epigenetic dysfunction of the retinal pigment epithelium in AMD

Maria Cristina Kenney[1,2,*], **Sonali Nashine**[1]

[1]Department of Ophthalmology, Gavin Herbert Eye Institute, University of California Irvine, Irvine, CA, USA

[2]Department of Pathology and Laboratory Medicine, University of California Irvine, Irvine, CA, USA

Abstract

Introduction: Modulation of epigenetic mechanisms that contribute to retinal development may render the eye susceptible to age-related macular degeneration (AMD). Progression of AMD involves alterations of epigenome such as CpG methylation and histone modifications, and study of the epigenetic regulation of molecular/ cellular pathways associated with AMD might identify target epigenetic markers for treatment of AMD.

Areas covered: In this review, we provide an overview of the influence of epigenetic factors on signaling pathways/ related genes associated with AMD, mainly hypoxia, angiogenesis, inflammation, complement, and oxidative stress; and discuss the critical role of microRNAs in AMD.

Expert Opinion: Better understanding of epigenetic-mediated and microRNA-mediated regulation of the AMD disease-related pathways would help to assess the risk of developing AMD besides providing valuable insight on potential target candidates for AMD therapy.

Keywords

AMD; Age-related macular degeneration; Epigenetics; DNA methylation; CpG methylation; Epigenetics; HDACs; Histone modifications; MicroRNAs; Precision medicine for AMD

1. INTRODUCTION

Age-related macular degeneration (AMD) is a major cause of vision loss in the elderly populations worldwide. While there are anti-VEGF (Vascular Endothelial Growth Factor) treatments for the advanced form with choroidal neovascularization (CNV), the dry form of AMD has no reliable therapies at this time. The pathophysiology of AMD is multifactorial

[*]**Corresponding Author:** Maria Cristina Kenney, Phone: (949) 824-7603, mkenney@uci.edu.

Declaration of Interests

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer Disclosures:

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

and involves multiple molecular pathways, including oxidative stress, cell signaling, inflammation/complement, angiogenesis, non-genetic factors, and degradative enzymes (Figure 1).

AMD has a genetic component, which has been well described by others. [1,2] The high-risk environmental factors include smoking, obesity, cardiovascular disease, and high fat diet with restricted antioxidants.[3,4,5,6] It is becoming more evident that epigenetic modification is also involved with AMD pathology. The epigenetics can modulate gene expression via DNA methylation at CpG sites and histone modifications (acetylation of lysine sites, methylation at arginine or lysine amino acids, phosphorylation of serine or threonine sites and lysine ubiquitination/sumoylation). The epigenetic changes result in unwinding of the DNA and altered transcriptional activity. Epigenetics can be modified through nutritional, environmental and pathological events and passed on to future generations.

AMD pathogenesis is known to involve epigenetic modifications such as DNA methylation, chromatin remodeling, and histone modifications, i.e., acetylation and methylation of lysine residues on histone proteins in DNA.[7,8]

DNA MethylTransferases (DNMTs) maintain methylation patterns in the genome and are critical to cellular homeostasis. DNMT1 catalyzes addition of methyl groups at CpG sites and is expressed in the retinal neurons. [9] DNMT1 is essential for differentiation of RPE and photoreceptor cells and for maintaining the levels of retinoid binding protein. DNMT1 deficiency leads to loss of retinal neurons. DNMT3A and DNMT3B play a vital role in de novo methylation and their expression levels decrease as the retina ages. Loss of DNMT3B causes abnormal development of RPE and retina.[10] Other effectors of DNA methylation in mammalian cells include MAT1A (Methionine AdenosylTransferase 1 Alpha), MAT2B, MBD2 (Methyl-CpG Binding Domain protein 2), and MBD4. In our recent studies, the transmitochondrial AMD RPE cells showed reduced expression of DNMT1 and MBD2; whereas the expression levels of DNMT3A, DNMT3B, MAT1A, MAT2B, and MBD4 were upregulated compared to the normal RPE cybrid cells. [11]

In this review, we describe the mechanism(s) of epigenetic regulation in genes/ markers associated with the pathogenesis of AMD.

2. OVERVIEW OF EPIGENETICALLY REGULATED SIGNALING PATHWAYS/ GENES ASSOCIATED WITH AMD:

2.1. Hypoxia and Angiogenesis

Loss of choriocapillaris and choroidal perfusion creates a hypoxic environment for the adjacent RPE cells, thereby inducing the release of angiogenic factors, including VEGF, which leads to neovascularization associated with wet AMD.[12] Hypoxia, a common pathophysiological characteristic of AMD, elicits oxidative stress, redox imbalance, and subsequent death of RPE cells.[13] HIFs (Hypoxia-Inducible Factors) are heterodimeric transcription factors which play crucial roles in transcriptional regulation and initiation of hypoxic cellular responses to altered oxygen tension in mammalian cells. The HIF-1 complex maintains oxygen homeostasis and is composed of two basic helix–loop–helix

(HLH)–PER–ARNT–SIM (bHLH–PAS) protein subunits. The first is HIF-1α, which is oxygen-regulated and exists at low levels in normoxia, and secondly is the constitutively expressed HIF-1β. The oxygen sensing ability of HIF-1α largely relies on the enzymes - prolyl hydroxylases 1–3 (PHD1–3) and asparagine hydroxylase factor inhibiting HIF (FIH). In normoxic cells, PHD1 is constitutively expressed and it hydroxylates the conserved proline residues i.e., Pro402 and Pro564 on HIF-1α. This allows the binding of von Hippel Lindau tumour suppressor protein (pVHL), the recognition motif of a ubiquitin E3 ligase complex, to HIF-1α and subsequently leads to ubiquitin-proteasome degradation of the HIF-1α subunits. The half-life of HIF-1α is less than 5 minutes in normoxic conditions. Under hypoxia, HIF-1α hydroxylation by PHD1 is suppressed and pVHL detaches from HIF-1α resulting in accumulation of HIF-1α. This allows HIF-1α to translocate from the cytosol to the nucleus where it dimerizes with HIF-1β and its cofactor p300/CBP. Following this, HIF-1α regulates gene expression by recognizing and binding to the HIF-1 Binding Site (HBS) localized in the Hypoxia-Responsive Element (HRE). This complex is located within the target gene promoters for transactivation of hypoxia response genes, which include the genes involved in angiogenesis and growth i.e., Angiopoietin 1, Angiopoietin 2, Endothelin-1, Nitric oxide synthase 2, and VEGF; the genes involved in proliferation and survival such as Erythropoietin and Transferrin; and the genes involved in energy metabolism such as Glucose transporter-1 and Glyceraldehyde-3-phosphate dehydrogenase, Hexokinase 1, Hexokinase 2.[14,15]

The VHL and PHD proteins which stabilize the HIF-1α transcription factor are regulated by their promoter methylation status (Figure 2, Table 1). In RPE cells, via the HIF pathway, cobalt chloride-induced hypoxia causes hypomethylation and upregulation of ten-eleven translocation (TET) genes namely TET1 and TET2.[16] The study shows that 5-azacytidine (DAC), an inhibitor of DNA methylation, demethylates the TET1 and TET2 promoters and reactivates gene expression in RPE cells, establishing the key role of epigenetic regulation of the hypoxia pathway via DNA methylation.

Hypoxia-induced upregulation and accumulation of VEGF, iNOS (the inducible form of nitric oxide synthase), and NO (nitric oxide) contribute to retinal vascularization, pathological angiogenesis and subsequent retinal damage in AMD. *VEGF* mRNA transcript and VEGF-A protein are upregulated in AMD RPE cells compared to normal control cells *in vitro*.[11] Upregulation of VEGF in AMD largely relies on the binding of HIF-1α to the HBS in the VEGF gene promoter. Aquaporin-4 (AQP4) which is a predominant integral membrane water channel protein in the retina, affects the physical interaction between HIF-1α and the HBS in the VEGF promoter. Absence of AQP4 prevents VEGF upregulation by hindering the hypoxia-induced HBS demethylation process. [17] Moreover, DAC-mediated demethylation results in downregulation of VEGF thereby bringing the VEGF level back to normal in human RPE cells and human retinal endothelial cells.[11,18] Treatment of AMD RPE cells with Trichostatin-A (TSA), an HDAC inhibitor, significantly reduces the levels of VEGF-A and HIF-α proteins compared to their untreated counterparts. TSA-induced anti-angiogenic effects are mediated via downregulation of VEGF and HIF-1α under hypoxic conditions both *in vitro* and *in vivo*. [19]

2.2. Oxidative stress

Reactive Oxygen Species (ROS) such as superoxide anion (O_2^-), singlet oxygen ($^1[O_2]$), and hydrogen peroxide (H_2O_2) are produced during oxidative cellular metabolism via the mitochondrial electron transport chain complexes. Alteration in redox homeostasis results in oxidative stress which is a primary contributor to AMD pathogenesis.[20] AMD patients have damaged mitochondria, higher ROS levels, and reduced antioxidant potential as evidenced by downregulation of mitochondria-specific antioxidant genes such as SOD2 (Superoxide Dismutase 2) and PRDX3 (Thioredoxin-dependent peroxide reductase) in transmitochondrial AMD RPE cybrid cells.[21,22] The phase II metabolic isoenzymes namely Glutathione S-transferases (GSTs) possess antioxidant properties and ROS scavenging potential. GSTs enable cellular detoxification by mediating the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates. [23,24] Decline in the transcript levels of GSTs in AMD cells enhances the cellular susceptibility to oxidative insults,[25] and retinal GSTs confer significant protection against oxidative stress-induced cellular damage.[26] Substantial decrease in the gene and protein levels of GSTM1 (GST mu1) and GSTM5 (GST mu5) was observed in the RPE/choroid and neurosensory retina in postmortem eyes of AMD patients compared to age-matched normal control subjects. Both GSTM1 and GSTM5 are expressed in the apical side of RPE, the nerve fiber layer, the outer plexiform layer, and the photoreceptor outer segments. CpG site bisulfite pyrosequencing established a strong correlation between hypermethylation of the *GSTM1* gene promoter in RPE/choroid (Table 1) and downregulation of *GSTM1* and *GSTM5* transcripts and proteins. It has been suggested that the GSTM1 promoter may act as a CpG island for the downstream GSTM5. This epigenetically regulated repression of GSTM1 and GSTM5 contributes to increased susceptibility to oxidative damage in the AMD retinas.[27]

2.3. Inflammation

Immunologic responses, including pro-inflammatory molecules, cytokines, complement activation, macrophage recruitment, and microglial activation, are implicated in the development of AMD disease.[28] Interleukin-17A (IL17A) signaling causes RPE cell toxicity in AMD retinas and is associated with inflammasome activation in the macula.[29] Both IL17A and IL17RC (Interleukin-17 Receptor C) are expressed in the macula of AMD patients.[30] In an examination of the non-genetic and environmental components of AMD, genome-wide DNA methylation patterns and modifications in histone proteins, the IL17RC expression was evaluated in the blood and retina of identical twins with discordant AMD phenotype. The promoter of *IL17RC* gene was hypomethylated, which led to significantly reduced expression of *IL17RC* gene and protein in the retina and choroid as well as peripheral blood of AMD patients.[31] Therefore, the study suggested that: a) epigenetic alterations in *IL17RC* gene contribute to AMD pathogenesis, and b) expression and promoter DNA methylation patterns of *IL17RC* may serve as biomarkers and potential candidates for AMD diagnosis and therapy respectively (Figure 1, Table 1).

2.4. Transforming Growth Factor-Beta (TGF-β)

signaling plays a vital role in oxidative stress-induced RPE cell senescence, RPE cell migration, induces VEGF, angiogenesis, and subsequent choroidal neovascularization in wet

AMD. Although the distribution of TGF-β isoforms (TGF-β1, TGF-β2, and TGF-β3) is heterogeneous as all three isoforms are expressed in vitreous and aqueous humor in the human eye, TGF-β2 is the predominantly expressed isoform in RPE cells, Bruch's membrane, and choroid. [32,33] This has been confirmed in various *in vitro* and *in vivo* studies of cultured RPE cells and primate ocular tissues. *SKI* is a proto-oncogene that represses TGF-β signaling by interacting with SMADs (acronym for *Caenorhabditis elegans SMA ("small" worm phenotype) and Drosophila MAD ("Mothers Against Decapentaplegic*") genes). In RPE cells, enhanced TGF-β signaling, which contributes to complement activation, corresponds to lower expression levels of *SKI* (Figure 3, Table 1). Genome-wide DNA methylation profiling using bisulfite-pyrosequencing in RPE cells obtained from AMD and normal human donor eyes revealed: a) significantly decreased expression of *SKI* gene in AMD RPE compared to normal RPE, b) differential methylation of CpG loci and differentially methylated locus cg18934822 in the *SKI* gene, c) enhancer signature H3K4me1 in the cg18934822 region and the CpG locus of *SKI* gene , and d) a differentially methylated locus cg22508626 within the gene GTF2H4 (General Transcription Factor IIH Subunit 4). It was speculated that alterations in DNA methylation within the *SKI* gene promoter result in complement dysfunction in AMD.[34]

2.5. *ARMS2* (Age-Related Maculopathy Susceptibility 2) gene

encodes a ~11.4 kDa and ~107 amino acids long secreted protein that is a component of the choroidal extracellular matrix of the eye. The 10q26 region of the *ARMS2* gene and the SNP rs10490924 are high-risk locus for AMD. Mutations in the *ARMS2* gene are associated with pathogenesis of AMD. Genome Wide Association Studies (GWAS) and methylation profiling of retina and peripheral blood samples from neovascular AMD patients and normal controls identified differential methylation levels of CpG sites in the *ARMS2* gene promoter, which correlated with the AMD risk genotype SNP rs10490924 (Figure 3, Table 1).[35]

2.6. *PRSS50* (Serine Protease 50) gene

encodes a protein with proteolysis serine-type endopeptidase activity and threonine-type endopeptidase activity. Genome-wide methylation profiling identified small but consistent variation in DNA methylation in the *PRSS50* gene. In the AMD blood and retina samples there was hypermethylation of three CpG islands in the 34 bp promoter region of the *PRSS50* gene compared to normal control samples (Figure 3, Table 1).[35]

2.7. *SOD2* (mitochondrial Superoxide Dismutase 2) gene

is known to protect RPE cells from oxidative stress and apoptosis. Substantive epigenetic modulation via histone modification in the *SOD2* gene was observed in hyperglycemic human AMD donor retinas. Significant decline in methylation of histone H3 lysine 4 (H3K4me2), enhanced methylation of histone H4 lysine 20 (H4K20me3), and increased acetylation of histone H3 lysine 9 (H3K9) were demonstrated in *in vivo* (Figure 3, Table 1). [36]

2.8. Matrix Metalloproteinases (MMPs)

are calcium-dependent endopeptidases that contribute to the degradation of extracellular matrix proteins and processing of bioactive molecules. MMPs play a key role in cell adhesion, migration, differentiation, proliferation, apoptosis, and angiogenesis. Both the MMP-2 (−1306 C/T) polymorphism and the SNP rs243865 CT are associated with the development of wet AMD in males less than 65 years of age.[37] MMP-9 was found to be upregulated in the plasma and aqueous humor of wet AMD patients. Logistic repression analyses revealed that (a) AMD patients had higher frequency of the MMP-9 (−1562) C/C genotype compared to controls, and (b) MMP-9 SNP rs3918242 (C–4T) was critical in the pathogenesis of AMD. [38,39] When human RPE cells were exposed to amyloid-β (a component of drusen in AMD) there was upregulation of MMP-9, which degraded the tight junction proteins and reduced the integrity of the epithelial barrier [40] (Figure 4, Table 1). The breakdown of the RPE barrier contributes to local inflammation, which is a hallmark of AMD. Therefore, regulation of MMP-9 is important for RPE cell health and stability. DNA methylation mediated epigenetic regulation of MMP-9 has been observed in the hippocampus as well.[41] Moreover, the 5-methylcytosine levels are drastically reduced in the promoter region of retinal MMP-9, suggesting that DNA methylation of the promoter plays a vital role in regulating the transcription of the *MMP-9* gene.[42]

2.9. Complement Factor H (CFH)

is a key inhibitor of the alternative complement pathway, and the Tyr402His mutation in the *CFH* gene is high-risk for AMD pathology. Significant downregulation of the *CFH* transcript and protein observed in AMD RPE cells has been associated with complement activation.[43] CpG methylation in *CFH* gene promoter regulates its expression and function (Figure 3, Table 1).[44]

2.10. NF-κB

(Nuclear Factor Kappa-light-chain-enhancer of activated B cells) is a transcription factor and a regulator of innate immune responses in aging. Activation of NF-κB and formation of glycoxidation product are associated with AMD disease pathology and neurodegeneration (Figure 1, Table 1). [45, 46]

2.11. Trichostatin A (TSA)

is a pan-HDAC inhibitor of mammalian class I, II, and IV HDACs. It chelates the zinc ion leading to HDAC inhibition and subsequently to cell cycle arrest and apoptosis.[47] In AMD and RPE cells, TSA downregulates VEGF, suppresses HIF-1α, and inhibits angiogenic effects. [48, 49]

2.12. SLC16A8

(Solute Carrier Family 16 Member 8) gene encodes MonoCarboxylate Transporter 3 (MCT3), a proton-coupled monocarboxylate transporter that is specifically and highly expressed in the RPE. MCT3 is often referred to as the lactate shuttle since it facilitates the transport of lactate across the RPE and out of the retina via choroidal circulation, thereby contributing to the maintenance of metabolic and ionic homeostasis of the outer retina.

Deletion of MCT3 leads to significant decline in subretinal space pH and altered visual function.[50] Multivariate logistic regression analysis revealed that the SNP rs8135665 (C/T) at locus 22q13.1 in the *SLC16A8* gene is high risk for AMD.[51] It has been reported that DNA methylation can alter lactate transport by inhibiting MCT3, and addition of 5-aza-2-deoxycytidine restored MCT3 activity and lactate transport.[52]

2.13. COL8A1

(Collagen Type VIII Alpha 1 Chain) gene encodes a short chain collagen which is a key component of the Descemet's membrane of the corneal endothelium and blood vessel endothelial cells, and is involved in extracellular matrix remodeling and the angiogenic activity of endothelial cells.[53] The SNP rs13081855 (G/T) at locus 3q12.1 in the *COL8A1* gene is high-risk for AMD.[51] Another lead variant i.e., rs140647181 (T/C) in the *COL8A1* gene is high-risk for AMD. It was recently reported that CpG site methylation in the *COL8A1* gene is regulated by the *OTX2* gene.[54]

2.14. TIMP3

(Tissue Inhibitor of MetalloProteinases 3) gene that encodes inhibitors of matrix metalloproteinases that cause degradation of the extracellular matrix. *TIMP3* gene SNP rs5749482 (C/G) at locus 22q12.3 shows significant association with AMD etiopathology.[51] Mutations in *TIMP3* are associated with autosomal dominant fundus dystrophy.[55] It has been reported that *TIMP3* gene expression is strongly influenced by histone modifications and promoter site DNA methylation in various tissues.[56,57]

2.15. APOE

(Apolipoprotein E) is a polymorphic susceptibility gene for AMD that encodes a key apolipoprotein of the chylomicron which is a ligand for LDL receptor and is essential for the normal catabolism of triglyceride-rich lipoprotein constituents and preserves the integrity of neuronal cell membranes.[58] Subretinal inflammation in AMD pathology is attributable to APOE isoforms.[59] The SNP variant rs4420638 (A/G) at *APOE* gene locus 19p13.2 is considered high-risk for AMD.[51] The SNPs rs429358 and rs7412 are known to reduce binding affinity to the LDL receptor.[60] However, the epsilon 4 allele of *APOE* gene is found at a very low frequency in AMD suggesting its role as a potential protective factor in AMD.[61] The *APOE* gene is differentially methylated in neurodegenerative diseases.[62,63]

3. Non-genetic factors in AMD:

Aging in conjunction with environmental factors such as smoking status and dietary habits constitute the non-genetic factors that account for 10 % of AMD.[64] Body mass index and smoking are the key non-genetic risk factors for AMD. Obesity is associated with high incidence of AMD.[65]

4. MicroRNA-mediated regulation in AMD

Regulation of gene expression via non-coding microRNAs constitutes a crucial part of epigenomics. MicroRNAs are crucial regulators of cellular functioning and recent studies

have revealed that circulating microRNAs carried by exosomes/microvesicles in the serum or blood plasma of patients serve as potential biomarkers of AMD. Significantly higher levels of miR-661 and miR-3121 were found in dry AMD patients whereas wet AMD patients showed significantly elevated levels of miR-4258, miR-889, and Let-7 microRNAs.[66] Let-7 microRNAs are strongly expressed in RPE ells and vascular endothelial cells. Inhibition of hypoxia-responsive microRNAs such as Let-7, miR-103, and miR-107, which are induced by *HIF-1α* and strongly expressed in vascular endothelial cells, reduces hypoxia-induced angiogenesis via translational desuppression.[67] Furthermore, three circulating microRNAs i.e., hsa-mir-301–3p, hsa-mir-361–5p, and hsa-mir-424–5p were found to be differentially secreted in neovascular AMD compared to controls. Further analysis of the genes regulated by these 3 microRNAs revealed the involvement of canonical TGF-β and MTOR pathways in neovascular AMD pathogenesis.[68] In addition, specific SNPs (Single Nucleotide Polymorphisms) in microRNA target-encoding loci of complement genes, ARMS2 gene, and MHC (Major Histocompatibility Complex) genes were recently identified, thereby indicating the ability of these SNPs to affect miRNA-mRNA pairings in loci implicated in the etiology of AMD. This study revealed elevated levels of hsa-miR 155–5p and hsa-let-7a/b/d-5p in AMD retinas; and decreased levels of hsa-miR-152 in AMD vitreous.[69]

MiR-146A:

MiR-146A SNP rs2910164 (C/G) is considered high risk for AMD. MiR-146A enhances inflammatory signaling and immune responses in neovascular AMD via interaction with its target genes i.e., *CFH, IL-6, IRAK1* (Interleukin 1 Receptor Associated Kinase 1), and *HTRA2* (High Temperature Requirement Protein A2, a mitochondrial serine protease).[70] Moreover, hsa-miR-146a-5p is known as a particularly relevant biomarker for AMD because: 1) it shows approximately 100-fold higher expression in choroid-RPE compared to neural retina;[71] 2) it is overexpressed in AMD retinas, shows 5-fold higher expression in the vitreous humor, and its plasma concentrations are 2.5-fold higher compared to age-matched controls; 3) the 3'UTR of CFH gene harbors a highly conserved and polymorphic seed pairing site for hsa-miR-146a-5p.[72,73]

MiR-27A:

Strafella *et al* demonstrated significant association between AMD risk and miR-27A SNPs rs11671784 (G/A) and rs895819 (T/C).[70] MiR-27A plays a key role in angiogenesis and choroidal neovascularization by targeted repression of the anti-angiogenic proteins SPRY2 (Sprouty2) and SEMA6A (Semaphorin6A), thereby facilitating the migration and proliferation of endothelial cells. In addition, miR-27A targets APBB2 (Amyloid beta A4 precursor protein-binding family B member 2) protein, which is involved in processing of Amyloid Precursor Protein (APP) into mature Amyloid-β protein in retinal and neuronal cells. Furthermore, mir-27A also targets VEGFC and PPARγ (Peroxisome Proliferator Activated Receptor Gamma) to regulate inflammatory and angiogenic responses, subsequently contributing to the etiopathology of AMD.

Several angiomiRs i.e., microRNAs that influence the process of angiogenesis have been identified and implicated in neovascular AMD. For instance, miR-210 regulates hypoxia-

mediated angiogenesis and cellular metabolism.[74] Similarly, miR-126 in response to pro-angiogenic factors triggers MAPK and PI3K signaling pathways; miR-126 knockout mice show delayed retinal vascularization and development.[75,76] Lin et al demonstrated that miR-23a, which regulates angiogenesis and choroidal neovascularization, protects RPE cells against hydrogen peroxide-induced injury via Fas-targeting.[77] Overexpression of miR-31 or miR-150 represses laser-induced choroidal neovascularization *in vivo*.[78]

DNA methylation directly regulates microRNA biogenesis thereby affecting microRNA functions; microRNAs are highly expressed, and sequence conserved when the regions flanking the microRNA coding sequence are highly methylated.[79] Loss of DNA methylation leads to upregulation of miR-21 and miR-146b and subsequent disruption of biological functions and transcriptional regulation.[80] Similarly, hypomethylation of miR-124–2 and miR-184 was considered a prognostic risk factor for breast cancer patients.[81]

EXPERT OPINION

In summary, the pathological events associated with AMD involve many different pathways, each regulated through complex interactions of genetic factors and environmental influences. Overriding these factors are the epigenetics, which can be changed during a person's lifetime due to stressors and nutrition or inherited from previous generations. The desire to understand further the epigenetic regulation of the AMD disease-related pathways is in part driven by the therapeutic opportunities for future drug development to block or inhibit the specific AMD pathways and slow or prevent the progression of this major disease of the aging eye.

The role of epigenetics in development of diseases is just being explored. A decade ago, the research community believed that sequencing of the entire human genome would provide the understanding of human diseases. However, it quickly became apparent that it is much more complicated, and the field of epigenetics began to burgeon. Epigenetics are mechanisms for gene regulation through DNA methylation and histone modifications that can result in phenotype changes, some of which are inheritable. While the underlying DNA sequence remains unchanged, the epigenetic modifications occur by methylation of CpG dinucleotides, often within promoter regions, and/or histone modifications (acetylation of lysine sites, methylation at arginine or lysine amino acids, phosphorylation of serine or threonine sites and lysine ubiquitination/sumoylation) that result in DNA unwinding and increased transcription activities. These types of regulation are critical for development, homeostasis and disease progression. By understanding the epigenetics of cell types, tissues and molecular pathways, it will open the possibilities of creating treatments to reverse the problems when the epigenetic regulation is abnormal.

The genetics and environmental risk factors for age-related macular degeneration (AMD) have been well-characterized and pathways include Oxidative Stress, Cell Signaling, Inflammation, Angiogenesis, Degradative Enzymes and Non-genetic Factors. Recent studies have shown that many markers in these pathways are regulated by epigenetics. For example, promoter CpG methylation regulates antioxidant enzymes, *SOD2* and *GPX1*; degradative enzymes *PRSS50*, *MMP9* and *ARMS2*; complement inhibitor, *CFH*; inflammation inhibitor,

SKI; and angiogenesis markers, *VEGF*, *PHD* and *VHL*. Histone modification regulates expression levels for *NFκB* and *SKI*.

Investigations using transmitochondrial AMD cybrid cell lines showed altered levels of methylation-related and acetylation-related RNAs/proteins. As evidence accumulates that epigenetics play a critical role in AMD, this provides an excellent opportunity to characterize target pathways and genes for future drug treatments. There are already effective FDA approved HDAC inhibitors to block histone deacetylation and alter gene transcription. Moreover, the DNMT nucleoside inhibitors effectively block promoter regions associated with pathways known to be involved with AMD. The field of cancer therapies are by far leading the way with drugs that specifically target the pathways of acetylation. For example, 1) HDAC inhibitors such as Trichostatin A/Vorinostat (class I, II, IV HDACs), Belinostat (class I, II HDACs), Panobinostat (pan-HDAC inhibitor), Romidepsin/ (class I HDACs), and/or 2) methylation inhibitors: DNMT inhibitors such as Azacitidine/Vidaza (5-azacitidine) and Decitabine/Dacogen (5-aza-2'-deoxycytidine). These drugs are currently in cancer clinical trials and show promise. Similar approaches should be undertaken for AMD, beginning with *in vitro* studies using primary AMD RPE cells, AMD cybrids and/or retinal cell lines stressed with known AMD stressors (i.e. amyloid-beta, 7-ketocholesterol, hypoxia) to determine whether inhibitors of HDACs and/or DNMTs might slow or reverse apoptosis and cell death. Then pre-clinical *in vivo* approaches using retinal degeneration animal models treated with combinations of HDAC inhibitors should be investigated. The family of inhibitors block different classes of HDACs so the outcomes might vary depending upon which pathways are targeted.

In summary (Figure 5), major disease-related pathways, such as angiogenesis and inflammation, are regulated in part by various microRNAs. These same pathways are also modulated by epigenetic events that up- or down-regulate genes within these pathways by chromatin remodeling, histone modifications or DNA methylation. New evidence is accumulating that these two regulatory events may interact with each other, but additional studies are required to more fully understand these mechanism(s). However, identifying multiple mechanisms to regulate disease-causing pathways offers tremendous opportunities to develop novel therapeutic agents.

Precision medicine:

Precision medicine refers to the clinical approach that optimizes patient care for treatment and prophylaxis of diseases by considering genomics, genetic and molecular profiling, environmental factors, and lifestyle for each patient. Precision medicine offers the advantage of personalized medical care by taking into account inter-patient variability that is lacking in a standard one-size-fits-all treatment regime.

Advances in pharmacoepigenomics and translational medicine have allowed the development of precision medicine and personalized care tailored to the varying characteristics of individual AMD patients. With the large amount of data being generated by Genome-Wide Association Studies (GWAS) and analyzed with Artificial Intelligence (AI) using machine learning algorithms, it is easier to correlate genomic sequences to the patient's phenotype on a sizeable scale.

For example, compared to the low-risk CC genotype, the TT genotype of *VEGFA* rs943080 (C/T) is associated with relatively poor response to anti-VEGF drugs. Therefore, higher and more frequent dosage of anti-VEGF drugs might be required for patients with TT genotype.[82,83] With respect to the *VEGFR2* gene, the CC genotype of rs2071559 shows better mean retinal sensitivity in response to anti-VEGF drug treatment.[84]

AMD patients have genetic variants of the CFH gene which lead to complement dysregulation. Complement therapeutics are focused on inhibiting the lectin, alternative, and classical pathways in order to restore regulation of complement system. Currently, complement-based therapeutics are being developed by a variety of pharmaceutical companies and are in clinical trials. For instance, a) POT-4 (Potentia), a protease inhibitor that targets C3 is currently in phase I clinical trials for wet AMD, b) LFG316 (Novartis), a monoclonal antibody targeting C5 is currently in phase II clinical trials for dry and wet AMD; c) Lampalizumab (Genentech), is an Antibody Fab fragment that targets complement factor D and is in phase III clinical trials for dry AMD therapy.[85]

The goal is to gain enough understanding of the epigenetic regulation of the harmful pathways involved in AMD pathogenesis (both dry and wet) so that targeted inhibition of epigenetic regulation might slow or reverse the retinal cell damage that leads to vision loss.

Acknowledgments

Funding:

This article is funded in part by the Arnold and Mabel Beckman Foundation, Discovery Eye Foundation, Edith and Roy Carver, Institute for Clinical and Translational Science (ICTS) at University of California Irvine, National Institutes of Health, National Eye Institute (NEI R01 EY0127363), Polly and Michael Smith, Research to Prevent Blindness and UCI School of Medicine. S Nashine is a recipient of the 2017 Genentech/ ARVO AMD Translational Research Fellowship and the 2016 RPB pilot research grant.

REFERENCES:

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers./

1. Ratnapriya R, Chew EY. Age-related macular degeneration-clinical review and genetics update. Clin Genet 2013; 84(2):160–6. [PubMed: 23713713]
2. DeAngelis MM, Owen LA, Morrison MA, et al. Genetics of age-related macular degeneration (AMD). Hum Mol Genet 2017; 26(R1): R45–R50. [PubMed: 28854576]
3. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. JAMA 1994; 272(18):1413–20. [PubMed: 7933422]
4. Mares-Perlman JA, Klein R, Klein BE, et al. Association of zinc and antioxidant nutrients with age-related maculopathy. Arch Ophthalmol 1996; 114(8):991–7. [PubMed: 8694736]
5. Seddon JM, Cote J, Davis N, et al. Progression of age-related macular degeneration: association with body mass index, waist circumference, and waist-hip ratio. Arch Ophthalmol 2003; 121(6):785–92. [PubMed: 12796248]
6. Chakravarthy U, Wong TY, Fletcher A, et al. Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. BMC Ophthalmol 2010; 10:31. doi: 10.1186/1471-2415-10-31. [PubMed: 21144031]
7. Portela A, Esteller M, 2010. Epigenetic modifications and human disease. Nat.Biotechnol 28 (10), 1057–68. [PubMed: 20944598]

8. Pennington KL, DeAngelis MM, 2016. Epigenetic mechanisms of the aging human retina. J. Exp. Neurosci 9 (2), 51–79. [PubMed: 26966390]

9. Rhee KD, Yu J, Zhao CY, et al. Dnmt1-dependent DNA methylation is essential for photoreceptor terminal differentiation and retinal neuron survival. Cell Death Dis 2012; 3, e427. [PubMed: 23171847]

10. Rai K, Jafri IF, Chidester S, et al. Dnmt3 and G9a cooperate for tissue-specific development in zebrafish. J. Biol. Chem 2010; 285 (6), 4110–4121. [PubMed: 19946145]

11. Nashine S, Nesburn AB, Kuppermann BD, et al. Age-related macular degeneration (AMD) mitochondria modulate epigenetic mechanisms in retinal pigment epithelial cells. Exp Eye Res 2019; 189:107701. [PubMed: 31226340] ** This study demonstrates the effects of AMD mitochondria (from AMD patients) on epigenetic regulation in human transmitochondrial retinal pigment epithelial (RPE) cells in vitro and suggests that retrograde signaling leads to mitochondria-nucleus interactions that influence the epigenetic status of the RPE cells.

12. Bhutto I, Lutty G. Understanding age-related macular degeneration (AMD): relationships between the photoreceptor/retinal pigment epithelium/Bruch's membrane/choriocapillaris complex. Mol Aspects Med 2012; 33(4):295–317. [PubMed: 22542780]

13. Nita M, Grzybowski A. The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxid Med Cell Longev 2016; 2016:3164734. [PubMed: 26881021]

14. Dengler VL, Galbraith M, Espinosa JM. Transcriptional regulation by hypoxia inducible factors. Crit Rev Biochem Mol Biol 2014; 49(1):1–15. [PubMed: 24099156]

15. Majmundar AJ, Wong WJ, Simon MC. Hypoxia-inducible factors and the response to hypoxic stress. Mol Cell 2010; 40(2):294–309. [PubMed: 20965423]

16. Alivand MR, Soheili ZS, Pornour M, et al. Novel epigenetic controlling of hypoxia pathway related to overexpression and promoter hypomethylation of TET1 and TET2 in RPE cells. J Cell Biochem 2017; 118(10):3193–3204. [PubMed: 28252217]

17. Pisani F, Cammalleri M, Dal Monte M, et al. Potential role of the methylation of VEGF gene promoter in response to hypoxia in oxygen-induced retinopathy: beneficial effect of the absence of AQP4. J Cell Mol Med 2018; 22(1):613–627. [PubMed: 28940930]

18. Xie M, Tian J, Luo Y, et al. Effects of 5-aza-2'-deoxycytidine and trichostatin A on high glucose- and interleukin-1?-induced secretory mediators from human retinal endothelial cells and retinal pigment epithelial cells. Mol Vis 2014; 20:1411–21. [PubMed: 25352747]

19. Kim MS, Kwon HJ, Lee YM, et al. Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. Nat Med 2001; 7(4):437–43. [PubMed: 11283670]

20. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiol Rev 2014; 94(3):909–50. [PubMed: 24987008]

21. Nashine S, Cohen P, Chwa M, et al. Humanin G (HNG) protects age-related macular degeneration (AMD) transmitochondrial ARPE-19 cybrids from mitochondrial and cellular damage. Cell Death Dis 2017; 8(7): e2951. [PubMed: 28726777]

22. Nashine S, Kanodia R, Nesburn AB, et al. Nutraceutical effects of Emblica officinalis in age-related macular degeneration. Aging (Albany NY) 2019; 11(4):1177–1188. [PubMed: 30792375]

23. Tew KD, Townsend DM. Glutathione-s-transferases as determinants of cell survival and death. Antioxid Redox Signal 2012; 17(12):1728–37. [PubMed: 22540427]

24. Birben E, Sahiner UM, Sackesen C, et al. Oxidative stress and antioxidant defense. World Allergy Organ J 2012; 5(1):9–19. [PubMed: 23268465]

25. Joshi PM, Franco M, Dubovy S, et al. Decreased expression of GSTP1 in the macula leads to AMD pathogenesis. Invest. Ophthalmol. Vis. Sci 2009; 50(13):2346.

26. Singh SV, Dao DD, Srivastava SK, et al. Purification and characterization of glutathione S-transferases in human retina. Curr Eye Res 1984; 3(11):1273–80. [PubMed: 6510009]

27. Hunter A, Spechler PA, Cwanger A, et al. DNA methylation is associated with altered gene expression in AMD. Invest Ophthalmol Vis Sci 2012; 53(4):2089–105. [PubMed: 22410570]

28. Copland DA, Theodoropoulou S, Liu J, et al. A perspective of AMD through the eyes of immunology. Invest Ophthalmol Vis Sci 2018; 59(4): AMD83–AMD92. [PubMed: 30025105]

29. Ardeljan D, Wang Y, Park S, et al. Interleukin-17 retinotoxicity is prevented by gene transfer of a soluble interleukin-17 receptor acting as a cytokine blocker: implications for age-related macular degeneration. PLoS One 2014; 9(4): e95900. [PubMed: 24780906]
30. Park Stanley, De Fen Shen Lai Wei, et al. IL-17A and IL-17RC expression in the macula of the human eye with Age-Related Macular Degeneration. Invest. Ophthalmol. Vis. Sci 2012; 53(14):1667.
31. Wei L, Liu B, Tuo J, et al. Hypomethylation of the IL17RC promoter associates with age-related macular degeneration. Cell Rep 2012;2(5):1151–8. [PubMed: 23177625]
32. Nagineni CN, Samuel W, Nagineni S, et al. Transforming growth factor-beta induces expression of vascular endothelial growth factor in human retinal pigment epithelial cells: involvement of mitogen-activated protein kinases. J Cell Physiol 2003; 197(3):453–62. [PubMed: 14566975]
33. Yu AL, Fuchshofer R, Kook D, et al. Subtoxic oxidative stress induces senescence in retinal pigment epithelial cells via TGF-beta release. Invest Ophthalmol Vis Sci 2009; 50(2):926–35. [PubMed: 19171648]
34. Porter LF, Saptarshi N, Fang Y, et al.. Whole-genome methylation profiling of the retinal pigment epithelium of individuals with age-related macular degeneration reveals differential methylation of the SKI, GTF2H4, and TNXB genes. Clin Epigenetics 2019; 11(1):6. [PubMed: 30642396] ** This study reports genome-wide methylation analysis of RPE in AMD and identifies novel targets for functional and future therapeutic intervention studies.
35. Oliver VF, Jaffe AE, Song J, et al.. Differential DNA methylation identified in the blood and retina of AMD patients. Epigenetics 2015; 10(8):698–707. [PubMed: 26067391] ** This study via genome-wide DNA methylation profiling of blood from AMD patients and controls demonstrates significant DNA methylation differences in novel genes that contribute to AMD development and progression.
36. 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. [PubMed: 23221071]
37. Liutkeviciene R, Vilkeviciute A, Borisovaite D, et al. Association of exudative age-related macular degeneration with matrix metalloproteinases-2 (−1306 C/T) rs243865 gene polymorphism. Indian J Ophthalmol 2018; 66(4):551–557. [PubMed: 29582818]
38. Jonas JB, Tao Y, Neumaier M, et al. Cytokine concentration in aqueous humour of eyes with exudative age-related macular degeneration. Acta Ophthalmol (Copenh) 2012; 90: 381–388.
39. Zeng J, Jiang D, Liu X, et al. Matrix metalloproteinases expression in choroidal neovascular membranes. Yan Ke Xue Bao 2004; 20:191–193. [PubMed: 15499730]
40. Cao L, Wang H, Wang F. Amyloid-?-induced matrix metalloproteinase-9 secretion is associated with retinal pigment epithelial barrier disruption. Int J Mol Med 2013; 31(5):1105–12. [PubMed: 23525277]
41. Zybura-Broda K, Amborska R, Ambrozek-Latecka M, et al. Epigenetics of Epileptogenesis-evoked upregulation of matrix metalloproteinase-9 in hippocampus. PLoS One 2016; 11(8):e0159745. [PubMed: 27505431]
42. Kowluru RA, Shan Y, Mishra M. Dynamic DNA methylation of matrix metalloproteinase-9 in the development of diabetic retinopathy. Lab Invest 2016; 96(10):1040–9. [PubMed: 27454437]
43. Nashine S, Chwa M, Kazemian M, et al. Differential expression of complement markers in Normal and AMD transmitochondrial cybrids. PLoS One 2016; 11(8): e0159828. [PubMed: 27486856]
44. Yang JD, Seol SY, Leem SH, et al. Genes associated with recurrence of hepatocellular carcinoma: integrated analysis by gene expression and methylation profiling. J Korean Med Sci 2011; 26(11):1428–38. [PubMed: 22065898]
45. Stirban A, Gawlowski T, Roden M. Vascular effects of advanced glycation endproducts: clinical effects and molecular mechanisms. Mol. Metab 2013; 3 (2), 94–108. [PubMed: 24634815]
46. Zeng HY, Tso MO, Lai S, et al. Activation of nuclear factor-kappaB during retinal degeneration in rd mice. Mol. Vis 2008; 14, 1075–1080 [PubMed: 18552981]
47. Yoshida M, Kijima M, Akita M, et al. Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. J. Biol. Chem 1990; 265 (28), 17174–17179. [PubMed: 2211619]

48. Xie M, Tian J, Luo Y, et al. Effects of 5-aza-2'-deoxycytidine and trichostatin A on high glucose and interleukin-1?-induced secretory mediators from human retinal endothelial cells and retinal pigment epithelial cells. Mol. Vis 2014; 20, 1411–1421. [PubMed: 25352747]

49. Kim MS, Kwon HJ, Lee YM, et al. Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. Nat. Med 2001; 7 (4), 437–443. [PubMed: 11283670]

50. Daniele LL, Sauer B, Gallagher SM, et al. Altered visual function in monocarboxylate transporter 3 (Slc16a8) knockout mice. Am J Physiol Cell Physiol 2008; 295(2):C451–7. [PubMed: 18524945]

51. Cascella R, Strafella C, Longo G, et al. Uncovering genetic and non-genetic biomarkers specific for exudative age-related macular degeneration: significant association of twelve variants. Oncotarget 2017; 9(8):7812–7821. [PubMed: 29487693]

52. Zhu S, Goldschmidt-Clermont PJ, Dong C. Inactivation of monocarboxylate transporter MCT3 by DNA methylation in atherosclerosis. Circulation 2005; 112(9):1353–61. [PubMed: 16116050]

53. Hopfer U, Fukai N, Hopfer H, et al. Targeted disruption of Col8a1 and Col8a2 genes in mice leads to anterior segment abnormalities in the eye. FASEB J 2005; 19(10):1232–44. [PubMed: 16051690]

54. Kaufman J, Wymbs NF, Montalvo-Ortiz JL, et al. Methylation in OTX2 and related genes, maltreatment, and depression in children. Neuropsychopharmacology 2018; 43(11):2204–2211. [PubMed: 30089883]

55. Schoenberger SD, Agarwal A. A novel mutation at the N-terminal domain of the TIMP3 gene in Sorsby fundus dystrophy. Retina 2013; 33(2):429–35. [PubMed: 23023527]

56. Shinojima T, Yu Q, Huang SK, et al. Heterogeneous epigenetic regulation of TIMP3 in prostate cancer. Epigenetics 2012; 7(11):1279–89. [PubMed: 23023649]

57. Su CW, Chang YC, Chien MH, et al. Loss of TIMP3 by promoter methylation of Sp1 binding site promotes oral cancer metastasis. Cell Death Dis 2019; 10(11):793. [PubMed: 31624299]

58. Klaver CC, Kliffen M, van Duijn CM, et al. Genetic association of apolipoprotein E with age-related macular degeneration. Am J Hum Genet 1998; 63:200–206. [PubMed: 9634502]

59. Levy O, Lavalette S, Hu SJ, et al. APOE isoforms control pathogenic subretinal inflammation in Age-Related Macular Degeneration. J Neurosci 2015; 35(40):13568–76. [PubMed: 26446211]

60. Siest G, Pillot T, Rgis-Bailly A, et al. Apolipoprotein E: an important gene and protein to follow in laboratory medicine. Clin Chem 1995; 41:1068–1086. [PubMed: 7628082]

61. Souied EH, Benlian P, Amouyel P, et al. The epsilon4 allele of the apolipoprotein E gene as a potential protective factor for exudative age-related macular degeneration. Am J Ophthalmol 1998; 125:353–359. [PubMed: 9512153]

62. Foraker J, Millard SP, Leong L, et al. The APOE gene is differentially methylated in Alzheimer's Disease. J Alzheimers Dis 2015; 48(3):745–55. [PubMed: 26402071]

63. Liu J, Zhao W, Ware EB, et al. DNA methylation in the APOE genomic region is associated with cognitive function in African Americans. BMC Med Genomics 2018; 11(1):43. [PubMed: 29739406]

64. Cascella R, Strafella C, Longo G, et al. Uncovering genetic and non-genetic biomarkers specific for exudative age-related macular degeneration: significant association of twelve variants. Oncotarget 2017; 9(8):7812–7821. [PubMed: 29487693]

65. Wang W, Gawlik K, Lopez J, et al. Genetic and environmental factors strongly influence risk, severity and progression of age-related macular degeneration. Signal Transduct Target Ther 2016; 1:16016. [PubMed: 29263899]

66. Szemraj M, Bielecka-Kowalska A, Oszajca K, et al. Serum MicroRNAs as potential biomarkers of AMD. Med Sci Monit 2015; 21:2734–42. [PubMed: 26366973]

67. Chen Z, Lai TC, Jan YH, et al. Hypoxia-responsive miRNAs target argonaute 1 to promote angiogenesis. J Clin Invest 2013; 123(3):1057–67. [PubMed: 23426184]

68. Grassmann F, Schoenberger PG, Brandl C, et al.. A circulating microrna profile is associated with late-stage neovascular age-related macular degeneration. PLoS One 2014; 9(9):e107461. [PubMed: 25203061] ** This study reports the role of circulating microRNAs in AMD by genome-wide miRNA expression profiling and replication analyses in neovascular AMD patients and age-matched controls.

69. SanGiovanni JP, SanGiovanni PM, Sapieha P, et al. miRNAs, single nucleotide polymorphisms (SNPs) and age-related macular degeneration (AMD). Clin Chem Lab Med 2017; 55(5):763–775. [PubMed: 28343170]
70. Strafella C, Errichiello V, Caputo V, et al. The interplay between miRNA-Related variants and Age-Related Macular Degeneration: EVIDENCE of association of MIR146A and MIR27A. Int J Mol Sci 2019; 20(7).
71. Karali M, Persico M, Mutarelli M, et al. High-resolution analysis of the human retina miRNome reveals isomiR variations and novel microRNAs. Nucleic Acids Res 2016; 44:1525–40. [PubMed: 26819412]
72. Bhattacharjee S, Zhao Y, Dua P, et al. MicroRNA-34a-mediated down-regulation of the microglial-enriched triggering receptor and phagocytosis-sensor TREM2 in age-related macular degeneration. PLoS One 2016; 11:e0150211. [PubMed: 26949937]
73. Menard C, Rezende FA, Miloudi K, et al. MicroRNA signatures in vitreous humour and plasma of patients with exudative AMD. Oncotarget 2016; 7:19171–84. [PubMed: 27015561]
74. Devlin C, Greco S, Martelli F, et al. miR-210: more than a silent player in hypoxia. IUBMB Life 2011; 63(2):94–100. [PubMed: 21360638]
75. Fish JE, Santoro MM, Morton SU, et al. miR-126 regulates angiogenic signaling and vascular integrity. Dev. Cell 2008; 15(2):272–284. [PubMed: 18694566]
76. Kuhnert F, Mancuso MR, Hampton J, et al. Attribution of vascular phenotypes of the murine Egfl7 locus to the microRNA miR-126. Development 2008; 135(24):3989–3993. [PubMed: 18987025]
77. Lin H, Qian J, Castillo AC, et al. Effect of miR-23 on oxidant-induced injury in human retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 2011; 52(9):6308–14. [PubMed: 21693609]
78. Shen J, Yang X, Xie B, et al. MicroRNAs regulate ocular neovascularization. Mol Ther 2008; 16(7):1208–16. [PubMed: 18500251]
79. Glaich O, Parikh S, Bell RE, et al. DNA methylation directs microRNA biogenesis in mammalian cells. Nat Commun 2019; 10(1):5657. [PubMed: 31827083]
80. Ortiz IMDP Barros-Filho MC, Dos Reis MB, et al. Loss of DNA methylation is related to increased expression of miR-21 and miR-146b in papillary thyroid carcinoma. Clin Epigenetics 2018; 10(1):144. [PubMed: 30454026]
81. Oltra SS, Pea-Chilet M, Vidal-Tomas V, et al. Methylation deregulation of miRNA promoters identifies miR124–2 as a survival biomarker in Breast Cancer in very young women. Sci Rep 2018; 8(1):14373. [PubMed: 30258192]
82. Zhao L, Grob S, Avery R, et al. Common variant in VEGFA and response to anti-VEGF therapy for neovascular age-related macular degeneration. Curr Mol Med 2013; 13(6):929–34. [PubMed: 23745581]
83. Dedania VS, Grob S, Zhang K, et al. Pharmacogenomics of response to anti-VEGF therapy in exudative age-related macular degeneration. Retina 2015; 35(3):381–91. [PubMed: 25635578]
84. Lazzeri S, Orlandi P, Piaggi P, et al.. IL-8 and VEGFR-2 polymorphisms modulate long-term functional response to intravitreal ranibizumab in exudative age-related macular degeneration. Pharmacogenomics 2016; 17(1):35–9. doi: 10.2217/pgs.15.153. [PubMed: 26653034] * This study reports IL-8 rs4073 and VEGFR-2 rs2071559 genotypes may serve as key molecular determinants to modulate final outcomes in neovascular AMD patients.
85. Yaspan BL, Williams DF, Holz FG, et al. Targeting factor D of the alternative complement pathway reduces geographic atrophy progression secondary to age-related macular degeneration. Sci Transl Med 2017; 9(395).

Article Highlights:

- Epigenetic dysregulation underlies the pathology of AMD.
- Study of epigenetically regulated AMD-associated markers may provide crucial information for identification of new potential candidates for AMD therapy.
- Key molecular/cellular pathways affected by epigenetic factors include, but are not limited to, hypoxia, angiogenesis, inflammation, and oxidative stress.
- MicroRNAs are regulated by DNA methylation and play a key role in AMD pathogenesis.

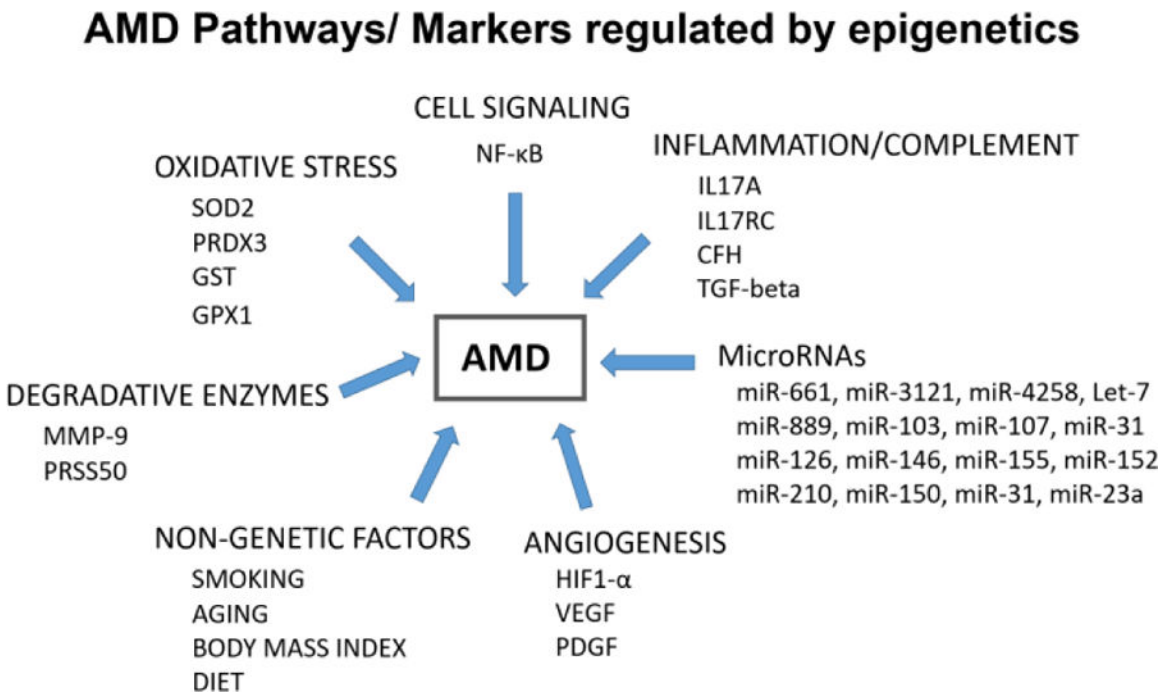

FIGURE 1.
Schematic diagram shows the epigenetically regulated AMD pathways and markers.

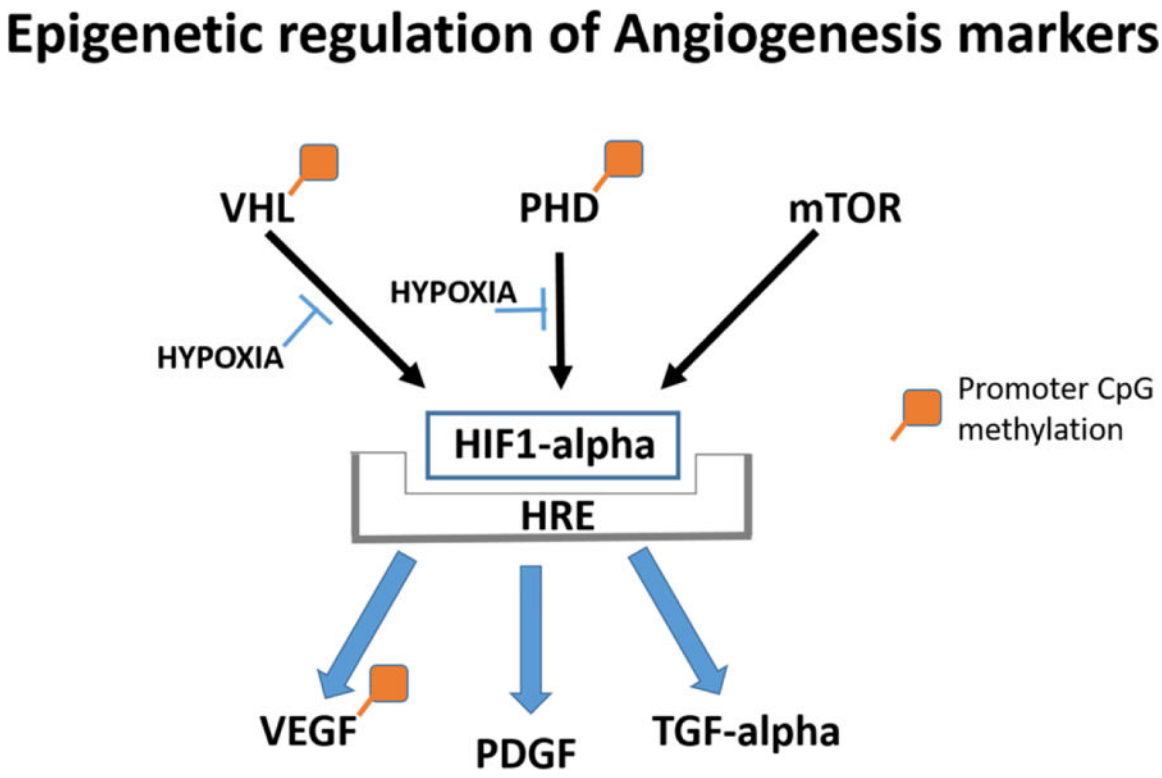

FIGURE 2.
Schematic diagram shows the regulation of angiogenesis markers via epigenetics.

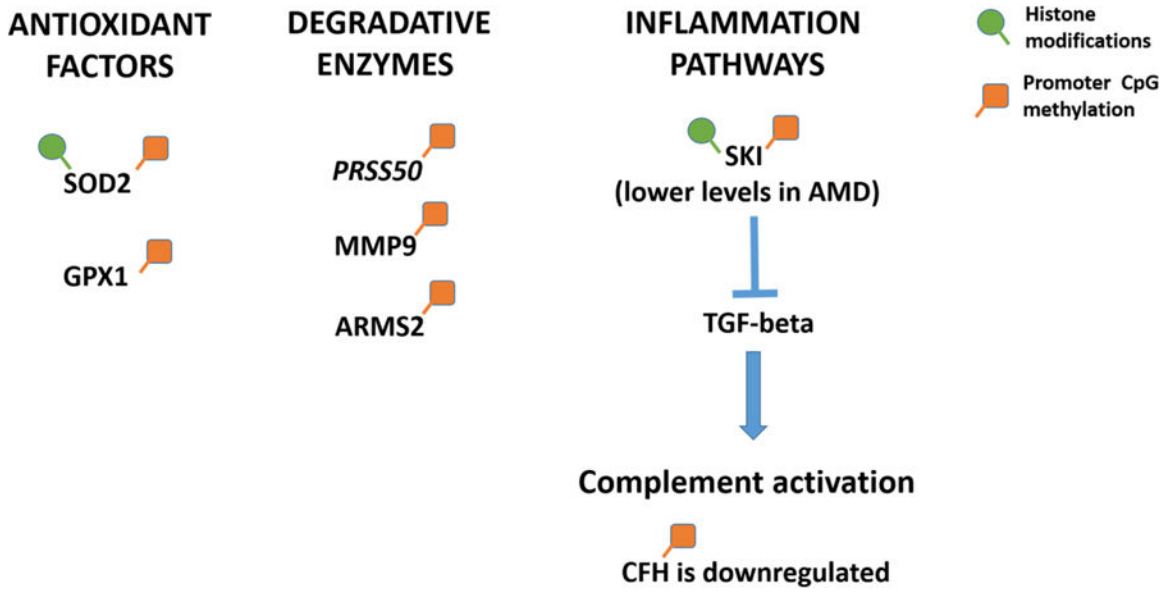

FIGURE 3.

Schematic diagram shows the sites of Histone modifications and Promoter CpG methylation in AMD associated molecular/cellular pathways.

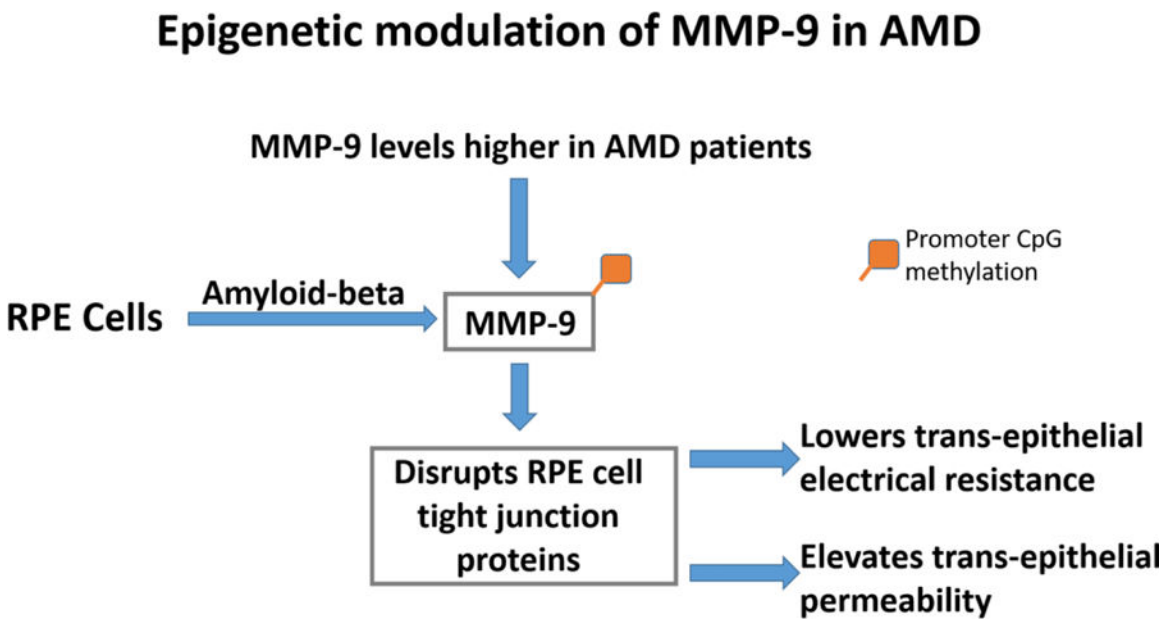

FIGURE 4.
Schematic diagram shows epigenetic modulation of MMP-9 in AMD.

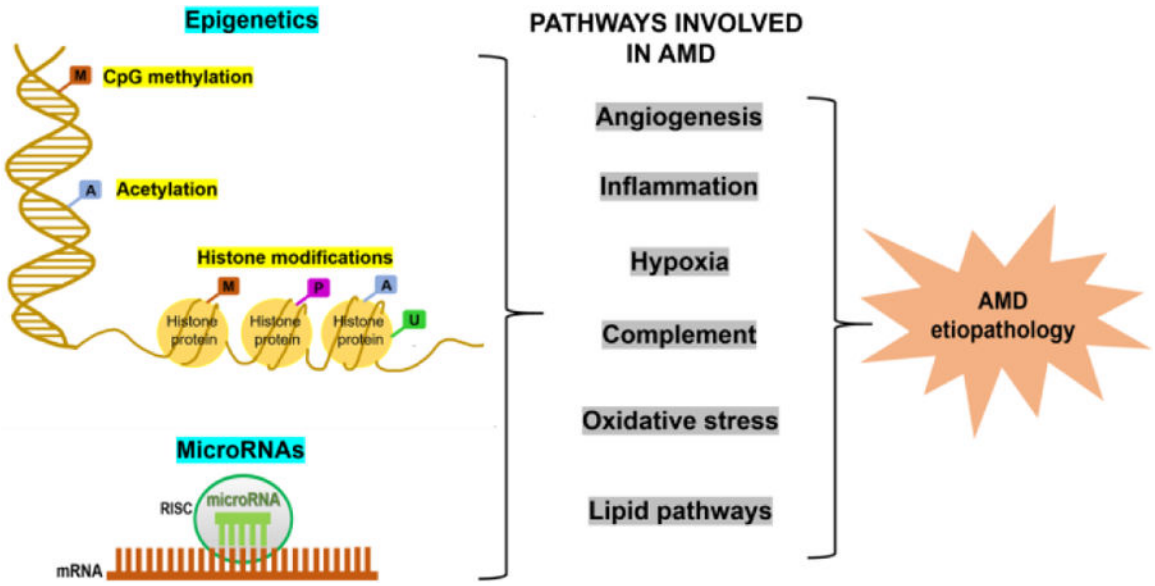

FIGURE 5.

Schematic summary of how epigenetic regulation and microRNAs contribute to the etiopathology of AMD.

Expert Rev Ophthalmol. Author manuscript; available in PMC 2021 March 16.

Table 1

This table lists the Epigenetic regulation mechanism(s) in Genes associated with AMD.

	Genes associated with AMD	Gene function in AMD/ RPE cells	Epigenetic Regulation Mechanism(s)
1	***HIF-1α*** (Hypoxia-Inducible Factor-1 alpha)	Transcriptional regulation and initiation of hypoxic cellular response in AMD.	*HIF-1α* stabilization is regulated by the promoter methylation status of VHL and PHD proteins.
2	***VEGF*** (Vascular Endothelial Growth Factor)	A signaling protein involved in angiogenesis and vasculogenesis in neovascular AMD.	• Methylation status of the HIF-1α- binding site in the *VEGF* gene promoter. • Expression of *VEGF* receptor genes is influenced by DNA methylation.
3	***CFH*** (Complement Factor H)	• Inhibitor of complement • SNP CFH rs10611770 (Tyr402His) variant is high-risk for AMD and associated with retinal drusen formation in AMD.	CpG methylation in the *CFH* gene promoter.
4	***IL17A/ IL17RC*** (InterLeukin 17A/ Receptor C)	• Inflammasome activation in macula. • RPE cell toxicity in AMD retinas.	DNA methylation patterns of *IL17RC* gene promoter.
5	***TGF-β*** (Transforming Growth Factor-Beta) ***SKI***	Signaling causes oxidative stress-induced RPE cell senescence, VEGF upregulation, and angiogenesis in AMD.	Histone modifications and methylation status of CpG loci of *SKI*, a proto-oncogene.
6	***MMP-9*** (Matrix Metalloproteinase-9)	An endopeptidase involved in degradation of extracellular matrix proteins and critical in AMD pathogenesis.	DNA methylation in the promoter of retinal MMP-9 gene.
7	***NF-κB*** (Nuclear Factor Kappa-light-chain-enhancer of activated B cells)	Transcription factor associated with retinal degeneration and glycoxidation product formation in AMD pathogenesis.	Methylation of arginine and lysine residues in the p65 subunit of NF-κB regulates its activation and expression.
8	***SOD2*** (Superoxide Dismutase 2)	Antioxidant activity that protects RPE cells from oxidative stress and apoptosis.	• CpG methylation. • Histone H3 and H4 modification via methylation and acetylation.
9	***PRSS50*** (Serine Protease 50)	Proteolysis serine-type endopeptidase and threonine-type endopeptidase activity associated with risk of AMD.	DNA methylation in CpG islands in promoter region of *PRSS50* gene.
10	***GSTM1*** (Glutathione S-transferases mu1)	Antioxidant role in cellular detoxification in RPE cells.	DNA methylation status of *GSTM1* gene promoter in the RPE influences the susceptibility to oxidative damage in AMD retinas.
11	***ARMS2*** (Age-Related Maculopathy Susceptibility 2)	High-risk locus for AMD disease pathology.	Methylation of CpG sites in the *ARMS2* gene promoter.
12	***GPX1*** (Glutathione Peroxidase 1)	Scavenges Hydrogen peroxide to nontoxic products thereby protecting RPE cells from oxidative damage.	DNA methylation.
13	***SLC16A8*** (Solute Carrier Family 16 Member 8)	Facilitates the transport of lactate across the RPE and out of the retina via choroidal circulation, thereby contributing to the maintenance of metabolic and ionic homeostasis of the outer retina.	DNA methylation.
14	***COL8A1*** (Collagen Type VIII Alpha 1 Chain)	• Extracellular matrix remodeling and the angiogenic activity of endothelial cells. • High-risk for AMD.	CpG site methylation.
15	***TIMP3*** (Tissue Inhibitor of MetalloProteinases 3)	Inhibitors of matrix metalloproteinases that cause degradation of the extracellular matrix.	Histone modifications and promoter site DNA methylation.

	Genes associated with AMD	Gene function in AMD/ RPE cells	Epigenetic Regulation Mechanism(s)
16	***APOE*** (Apolipoprotein E)	Normal catabolism of triglyceride-rich lipoprotein constituents and preserves the integrity of neuronal cell membranes. Subretinal inflammation in AMD pathology.	DNA methylation.