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Epigenetic modifications as potential therapeutic targets in age-related macular degeneration and diabetic retinopathy

Faith A.A. Kwa[1] and Thilini R. Thrimawithana[2]

[1] Discipline of Laboratory Medicine, School of Medical Sciences, RMIT University, Bundoora, VIC 3083, Australia
[2] Discipline of Pharmacy, School of Medical Sciences, RMIT University, Bundoora, VIC 3083, Australia

Recently, aberrant epigenetic modifications have been identified in the pathogenesis of the posterior eye diseases, age-related macular degeneration (AMD) and diabetic retinopathy (DR). This has led to the development of alternative therapies that can alter aberrant chromatin-remodelling processes involved in AMD and DR. These novel therapeutic agents could help to ameliorate the challenges associated with current treatments that are limited by variable patient response and disease heterogeneity. However, research on the use of epigenetic-based therapies in these diseases is relatively young and, therefore, preclinical studies that evaluate their mechanism of action, specificity and adverse effects are warranted.

Introduction

Epigenetics is the study of heritable changes in gene expression and function that are mediated by the activity of chromatin-remodelling enzymes, rather than by modifications in the underlying DNA sequence. In mammals, such enzymes are involved in a series of epigenetic mechanisms, namely DNA methylation and/or demethylation and histone deacetylation and/or acetylation [1]. During DNA methylation, a methyl group (−CH3) is added to the 5′ carbon of cytosine residues within the cytosine–guanine dinucleotides (CpG) by DNA methyltransferases (DNMT), resulting in the formation of 5-methylcytosine [2]. DNA demethylation is a process characterised by a series of catalytic events mediated by a variety of enzymes. For example, the Ten-eleven translocation family enzymes oxidise methylated cytosines, whereas the removal of methyl groups from the oxidised cytosines possibly occurs via thymine DNA glycosylase activity [3]. Histone acetylation is mediated by histone acetyltransferases (HATs) and involves the transfer of the acetyl group from acetyl coenzyme A to histone lysine residues. Histones can be deacetylated by histone deacetylases (HDACs) [4]. HDAC and DNMT activities lead to histone deacetylation and methylated chromatin, contributing to a 'closed' nucleosome formation (i.e. heterochromatin) that inhibits DNA transcription [5]. By contrast, an 'open' chromatin structure characterised by a widely spaced nucleosome (i.e. euchromatin) facilitates the binding of transcription factors to DNA and, hence, allows DNA transcription to occur [4,5]. This is mediated via DNA demethylation and HAT activity. Although chromatin remodelling is a naturally occurring process that regulates normal gene expression, diseases are often the resultant phenotype of aberrant chromatin-modifying processes and an imbalanced heterochromatin:euchromatin ratio.

Epigenetics research has progressed substantially since global DNA hypomethylation was first identified as a common feature of selective human tumours [6]. Thus, the detection of hypermethylated tumour suppressor genes and the dysregulation of HATs and HDACs in various malignancies has accounted for the atypical overproliferation, cell cycle regulation and apoptotic resistance of cancer cells [7]. More recently, abnormal epigenetic patterns have been reported to regulate various cellular and tissue processes, such as ageing, inflammation, immunomodulation and angiogenesis, and, hence, have been implicated in the pathogenesis of ocular disorders, including AMD and DR [8,9]. Thus, epigenetics research could provide new opportunities to explore the molecular basis of noninherited risk and environmental basis of diseases with complex pathogenesis, such as AMD and DR. This would provide fundamental insights into the design of improved treatment strategies.

Current treatment options for AMD and DR include laser photocoagulation and intraocular administration of vascular endothelial growth factor (VEGF) inhibitors and steroids [10,11]. However, because of the increasing prevalence and heterogeneity of these debilitating eye diseases, further molecular targets still need to be

Corresponding author:. Kwa, Faith A.A. (faith.kwa@rmit.edu.au)

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identified for the efficient management of both AMD and DR [11]. Emerging new therapies, such as epigenetic-based agents focussed on targeting regulators of inflammatory, angiogenic and oxidative damage pathways, could prevent and/or retard disease progression.

The role of epigenetics changes in the pathogenesis of AMD

It is well established that AMD is characterised by the presence of extra-retinal deposits of protein and lipid known as drusen that ultimately lead to pigmentary and visual disturbances [12]. Impaired phagocytosis of retinal debris and accumulation of lipofuscin might also be responsible for AMD-associated symptoms [13]. The pathogenesis of this disease can be divided according to 'dry' and 'wet' types. Age-progressive inflammatory damage and apoptosis of retinal pigment epithelium (RPE) and photoreceptors contribute to the atrophy and central vision loss in 'dry' AMD, whereas the central blindness in 'wet' AMD is caused by chronic hypoxia via mammalian target of rapamycin/hypoxia-inducing factor (mTOR/HIF), VEGF-induced choroidal neovascularisation, decreased tight and adherens junctions, and subsequent leakage of new blood vessels [14,15].

In a genome-wide association study (GWAS) on more than 17 000 patients with advanced AMD, Fritsche and colleagues identified seven new genomic loci that are linked to the regulation of complement activity, lipid metabolism, extracellular matrix remodelling and angiogenesis observed in AMD, namely collagen, type VIII, alpha 1 – filamin A interacting protein 1-like (*COL8A1–FILIP1*), immediate early response 3 – discoidin domain receptor tyrosine kinase 1 (*IER3–DDR1*), solute carrier family 16 (monocarboxylate transporter), member 8 (*SLC16A8*), transforming growth factor, beta receptor 1 (*TGFBR1*), RAD51 paralogue B (*RAD51B*), ADAM metallopeptidase with thrombospondin type 1 motif, 9 (*ADAMTS9*) and beta 1,3-galactosyltransferase-like *B3GALTL*. Other genetic risk factors of AMD include genes encoding age-related macular degeneration 1 (*ARMD1*), apolipoprotein E (*APOE*) and Complement Factor H (*CFH*); VEGF genes have also been reviewed [16]. Interestingly, both mitochondrial and nuclear DNA from RPE cells of patients with AMD showed increased oxidative damage, thereby indicating the role of defective or imbalanced redox enzymes in the pathogenesis of this disease [17]. Environmental risk factors that might contribute towards the elevated oxidative stress in AMD include smoking, low omega-3 diet, excessive retinal iron levels and ageing [18]. However, there are presently no molecular markers to monitor disease progression. It is speculated that both genetic and environmental factors could act synergistically to increase the risk of progression [15].

In view of the above, the key pathological features of AMD are caused by high oxidative stress, which is explained by the accumulation of oxidised polyunsaturated fatty acids and depletion of antioxidants (e.g. vitamin C) with age and long-term intense light exposure [19,20]. The oxidative damage can be marked by decreased mRNA and protein levels of detoxification enzymes, such as glutathione S-transferase (GST; e.g. GSTM1 and GSTM5) in RPE cells that have been correlated with total GSTM1 promoter hypermethylation [21]. Additional evidence supporting the role of aberrant epigenetic modifications is provided by Gnana-Prakasam *et al.*, who reported significant increases in mRNA expression of HDAC1, HDAC3, HDAC6, DNMT1 and DNMT3a mRNA expression in RPE cells of mice with excessive iron levels and, thus, are at a higher risk for AMD [22]. Furthermore, ageing is an important factor in alterations of DNA methylation and histone acetylation status that might exacerbate these undesired modifications, leading to the gene-silencing effects observed in AMD [23].

Studies on epigenetic mechanisms in ocular diseases only began during the past decade. One of the earlier studies reported that HIF-1α expression is downregulated by HDAC1 activity [24]. Interestingly, VEGF expression downregulates HDAC7 and promotes expression of genes involved in angiogenesis [25]. Inflammation is another hallmark of AMD and is triggered by changes in histone acetylation and methylation status that involves the production of inflammatory cytokines and autoinflammatory T cells [26]. For example, an increased level of interleukins (i.e. IL-17 and IL-22) was found in the serum of patients with AMD. IL-17 and IL-22 released from a subset of CD4+ helper T cells can promote IL-17 receptor C (IL17RC) promoter demethylation, which enhances IL17RC expression and, hence, further amplifies a chronic inflammatory response in the macula [27]. These observations suggest the importance of aberrant methylation patterns in the hyperactivity of the aged immune system associated with the disorder. Given that oxidative damage associated with AMD is influenced by the imbalance between hypermethylation and hypomethylation, diminished expression of redox enzymes and scavengers of reactive oxygen species (ROS) is observed, leading to reduced protection from antioxidants [21]. The cellular redox state of the retina as age progresses might involve the activation of a stress-responsive HDAC known as sirtuin 1 (SIRT1). SIRT1 triggers hypoxia and angiogenesis via upregulating the expression of HIF-2α, VEGF and erythropoietin [14,22]. Together with SIRT1-induced deacetylation of the p53 protein, the reduced expression of antiapoptotic genes sensitises aged RPE cells to apoptosis [13]. Clusterin, a major component of drusen deposits, was found to be more highly expressed in cultured RPE cells derived from patients with AMD compared with healthy donors of similar age because of hypomethylation of the clusterin promoter [12].

The use of chromatin-modifying agents in the treatment of AMD

DNMT inhibitors

DNMT inhibitors (DNMTIs) aim to block methylation, reactivate the expression of genes and reverse pathological processes. *In vitro* studies have shown the ability of the DNMTI, 5-aza-2′-deoxycytidine (AZA), to upregulate clusterin expression in RPE cells via hypomethylation of the CpG islands in its promoter region [12]. This observation suggests the need for agonists to enhance DNA methylation of the clusterin promoter, particularly during the early stages of AMD. Interestingly, Hellebrekers *et al.* reported the ability of AZA to inhibit angiogenesis in *in vitro* and *in vivo* tumour models [28]. Therefore, AZA might retard the neovascularisation in 'wet' AMD. The above findings suggest that the effective use of DNMTIs in AMD treatment will vary according to the stage and/or form of the disease.

HDAC inhibitors

There has been much clinical success in the use of HDACIs in eradicating the pathological processes (e.g. inflammation and angiogenesis) in various disorders, especially cancer [29]. Recently,

it was revealed that HDACIs have promising effects in animal models of inflammatory disease and might hold the key to AMD treatment [30]. The hydroxamic acid-derived HDACI, trichostatin A (TSA), interferes with chromatin-remodelling processes leading to the accumulation of hyperacetylated histones, suppresses inflammation by inhibiting the expression and production of inflammatory cytokines and chemokines, and reduces the capacity of dendritic cells to migrate to inflamed sites [31,32]. In a retinal ischaemic rat model of AMD, TSA was found to protect the retina from ischaemic damage and to inhibit the activity of matrix metalloproteinases (MMPs), which is responsible for the degradation of the Bruch's membrane, resulting in AMD pathogenesis [33].

Other HDACIs, including valproic acid (VPA), have been shown to inhibit angiogenesis and complement activation *in vitro* and *in vivo*, thereby attenuating the undesired neovascularisation and drusen formation characteristic of AMD [34]. Under hypoxic conditions, SIRT1 reduces and increases the expression of HIF-2α and VEGF proteins, respectively [14]. Sirtinol, an inhibitor of SIRT1, can reverse these changes and reduces choroidal endothelial cell proliferation and, hence, neovascularisation [14]. This observation has been further validated by reports that reveal the ability of HDACIs to influence the expression of a range of angiogenesis-related genes, such as angiopoietin 2 and endothelial cell nitric oxide synthase [35,36]. Some naturally occurring compounds have been proven to exhibit HDAC inhibitory properties. One example is L-sulforaphane (LSF), an extract derived from cruciferous vegetables, such as broccoli. LSF protects the retina by exerting antioxidative and anti-inflammatory effects via inhibition of HDACs, which induces the expression of redox proteins, including GST, nicotinamide adenine dinucleotide phosphate, quinone reductase and thioredoxin in RPE cells and attenuation of retinal cell apoptosis in response to various inflammatory stimuli (e.g. ischaemia-induced injury) [20,37]. Hence, LSF is a promising epigenetic-based therapeutic agent for early-stage AMD. Although there are no current clinical trials or US Food and Drug Administration (FDA)-approved HDACIs for AMD, a patent that specifies the use of hydroxamic acid-based HDACIs in the treatment of ocular neovascular or oedematous disease, including AMD and DR, has been published [38].

Other potential epigenetic-based therapies

13-*Cis* retinoic acid and some of its synthetic derivatives have been used as a supplement for eye health and they are known to delay visual disturbances because of their ability to inhibit the formation of 11-*cis* retinal in the RPE, a key step in the visual retinoid cycle. These compounds can alter the chromatin structure and modulate the expression of angiogenesis-related genes, such as those encoding thrombospondin and pigment epithelium-derived factor in human RPE cells, thus exerting antiangiogenic effects [39]. 9-*Cis* retinoic acid was also found to promote apoptosis of vascular endothelial cells without the presence of an intact RPE [40].

RPCs are multipotent and capable of self-renewal. This might favour the development of HDACIs and DNMTIs that target and promote the expression of genes involved in neurogenesis and gliogenesis, such as hairy and enhancer of split (Hes)-1, Hes-5, Hesr2: Hairy and enhancer of split-related 2, Mash1: Mammalian achaete scute homolog-1, mouseath (Math)-3, Math-5, neuronal differentiation (Neurod)-1 and Neurod-4, to enable regeneration of the damaged retina in AMD [41].

Antiapoptotic proteins, such as B-cell CLL/lymphoma 2 (Bcl-2) would also be an ideal drug target for AMD because their expression decreases with age [13]. Resveratrol, a compound found in the skin of red grapes, is reported to regulate HDAC activity and ultimately inhibit caspase 3 activation [13]. Therefore, future AMD treatment can involve the combined administration of Bcl-2-targeted drugs and resveratrol to restore the integrity and prevent apoptosis of the RPE.

The experimental methodology used to investigate the above-mentioned chromatin-modifying agents in this disease is summarised in Table 1 for comparative purposes.

The role of epigenetics changes in the pathogenesis of DR

DR is a microvascular complication of diabetes and the leading cause of blindness among adults [42]. It involves obstruction and increased permeability of retinal vessels, which leads to macular oedema during the nonproliferative phase (i.e. DME) and abnormal neovascularisation on the retinal surface during the proliferative phase (i.e. PDR). In advanced disease, retinal and vitreous haemorrhage, retinal detachment, fibrous proliferation and neovascular glaucoma are evident [42].

Poor glycaemic control, inflammation and neurodegeneration have been identified as the key contributing factors in the pathophysiology of DR. The mechanisms of cellular damage induced by hyperglycaemia include formation of advanced glycation-end products and activation of the aldose-reductase metabolic pathway, protein kinase C and ROS [42]. Despite the identification of several common genetic variants and the strong familial clustering of diabetes, the association between genetic factors and DR is yet to be fully elucidated [43]. More recently, epigenetics processes have been proposed to have a significant role in the pathogenesis of diabetic complications, familial clustering and metabolic memory in diabetes [44].

The role of histone modifications in diabetic complications has been verified. Results from a genotyping study on patients with type 1 diabetes mellitus ($N = 2991$) revealed an association between a polymorphism in a Histone 3 Lysine 9 (H3K9) selective methyltransferase gene (Suppressor of Variegation 3–9 Homolog 2; *SUV39H2*) and DR [45]. Syreeni *et al*. also defined the altered function of DNMT as an early event in diabetic vascular complications and suggested the role of *SUV39H2* in the hyperglycaemia-induced inflammatory response [45].

Epigenetic modification of *Sod2*, a gene that encodes mitochondrial superoxide (MnSOD), has also been implicated in the pathogenesis of DR. MnSOD has been shown to protect retinal endothelial cells from glucose-induced oxidative stress and apoptosis. This protective system is impaired in diabetes and is likely to promote the onset of DR [46]. Zhong and Kowluru reported that hyperglycaemia leads to a significant epigenetic alteration of *Sod2* with an increase in tri-methylation of histone H4 lysine 20 (H4K20me3) and acetylation of histone H3 lysine 9 (H3K9) in a rodent model of diabetes [47]. Their preclinical studies demonstrated a decrease in methylation of histone H3 lysine 4 (H3K4me2) at the *Sod2* gene. Additionally, a significant decrease in H3K4me2 at *Sod2* and *Sod2* gene expression in retinas obtained from human donor eyes with DR compared with nondiabetic donor eyes was observed. This study also demonstrated the irreversible nature of the methylation status

TABLE 1

Overview of experimental methodology used in studies investigating the use of chromatin-modifying compounds in the treatment of AMD and DR

Compound	Potential target disease	Experimental model	Dose	Route of administration	Refs
5-Aza-2′-deoxycytidine	AMD	Human RPE cells	10 MM	N/A	[11]
		Human umbilical vein endothelial cells	200 nM to 10 MM	N/A	[27]
		Mouse b.END5 brain endothelioma cells	0.1 MM to 10 MM	N/A	
		Mouse tumour model (C57BL/6; 6-week-old injected B16F10 mouse melanoma cells)	10 mg/kg administered daily for 7 days	Intraperitoneal	
Curcumin	DR	Streptozotocin-induced diabetic rats (Wistar albino)	1 g/kg body weight for 16 weeks	Oral	[59]
		Baculovirus-expressed recombinant full-length p300	50 mM, 100 mM, 200 mM and 300 mM	N/A	[58]
L-sulforaphane	AMD	4-Week-old BALB/C mice	0.5 mg per day up to 5 days	Intraperitoneal	[36]
		Human adult RPE cell line (ARPE-19)	5 mM	N/A	[19]
Minocycline	DR	Streptozotocin-induced diabetic rats (Lewis)	10 mg/kg administered five times per week for 10 weeks	Intraperitoneal	[48]
		Retinal Müller cell line	20 nM or 5 MM (with 30 mM glucose)	N/A	
		Retinal Müller cell line	10 mM, 25 mM, and 50 mM (with 25 mM glucose)	N/A	[57]
		Primary Müller cells	10 mM, 25 mM, 50 mM and 100 mM (with 25 mM glucose)	N/A	
Resveratrol	AMD	Primary human RPE cells	25 MM	N/A	[12]
Sirtinol	AMD	Choroidal endothelial cells (RF/6A)	200 mM	N/A	[13]
		Primary human RPE cells	25 mM and 50 MM	N/A	[12]
Trichostatin A	Retinal degenerative diseases (e.g. AMD, DR, glaucoma and retinal artery occlusion)	Brown Norway rats	2.5 mg/kg	Intraperitoneal: Day 0: 1 h before and 4 h after ischaemic injury Days 1, 2 and 3: twice daily	[32]
		Primary astrocytes	100 nM	N/A	
	Inflammatory diseases	Murine dendritic cells (bone marrow derived)	10 nM, 50 nM or 500 nM for 1 h	N/A	[30]
Valproic acid	Angiogenesis-related disorders (e.g. AMD and DR)	Human umbilical vein endothelial cells	0.25 mM to 1 mM	N/A	[33]

despite re-establishment of good glycaemic control in diabetic rodent models, thereby illustrating the association of histone methylation with the metabolic memory phenomenon [48].

Reduced acetylation of retinal histone 3 (H3) has also been demonstrated using a rat model of diabetes [49]. Given that a persistent increase in HDAC activity and compromised H3 and H3K9 acetylation was observed after termination of a poor glycaemic control for 6 months, this finding supports the role of histone modifications in metabolic memory. By contrast, Kadiyala *et al.* found a significant increase in retinal H3 and H4 acetylation in diabetic rats [50]. Such conflicting evidence could be attributed to the inconsistent duration of hyperglycaemia in diabetic animal models [49,50]. This warrants further investigation into the role of HDAC and HAT activity in the development of DR.

Proteolytic enzymes, such as MMPs, were reported to be elevated in various tissues of patients with diabetes. Kowluru *et al.* showed that ablation of the MMP-9 gene ameliorated diabetic rodents from developing DR [51]. Zhong and Kowluru investigated the involvement of epigenetics in the expression of MMPs in DR and showed an increase in the activity of lysine-specific demethylase 1 (LSD1) in the retina [52]. The authors also demonstrated a reduction in histone H3 dimethyl lysine 9 (H3K9me2) and an increase in acetyl H3K9 at the retinal MMP-9 promoter in rodents. Additionally, similar epigenetic alterations in the MMP-9 promoter region have been identified in human donor eyes with DR [52].

Hypermethylation of the CpG sites at the regulatory region of DNA polymerase gamma (POLG1) might also dictate DR

progression [53]. POLG1 is involved in DNA transcription and the resulting epigenetic modification leads to impairment of the mitochondrial DNA replication system and subsequent apoptosis of retinal capillary cells. Tewari *et al.* also demonstrated a significant increase in DNMT activity in the retinal nuclear fraction of the rodents with DR [54].

The use of chromatin-modifying agents in the treatment of DR

HDAC inhibitors

Recent studies have indicated HDACs as potential therapeutic targets for various diseases, including neurodegenerative disorders, autoimmune disorders and diabetes mellitus [55]. Fan *et al.* reported that HDAC2, the primary isoform localised in the inner retinal layers, represents nearly 35% of the total HDAC activity in the retina [56]. *In vivo* studies demonstrated that selective reduction in HDAC2 activity via genetic mutation ($Hdac2^{+/-}$ mice) significantly reduced the ischaemic injury to the retina compared with wild type controls ($Hdac2^{+/+}$ mice) [56]. Crosson *et al.* demonstrated that intraperitoneal administration of TSA 1 h before induction of retinal ischaemia in rats provided significant neuroprotection and preserved the normal morphology of rat retinas compared with retinas in the control group [33]. Similarly, Zhang *et al.* reported a reduction in

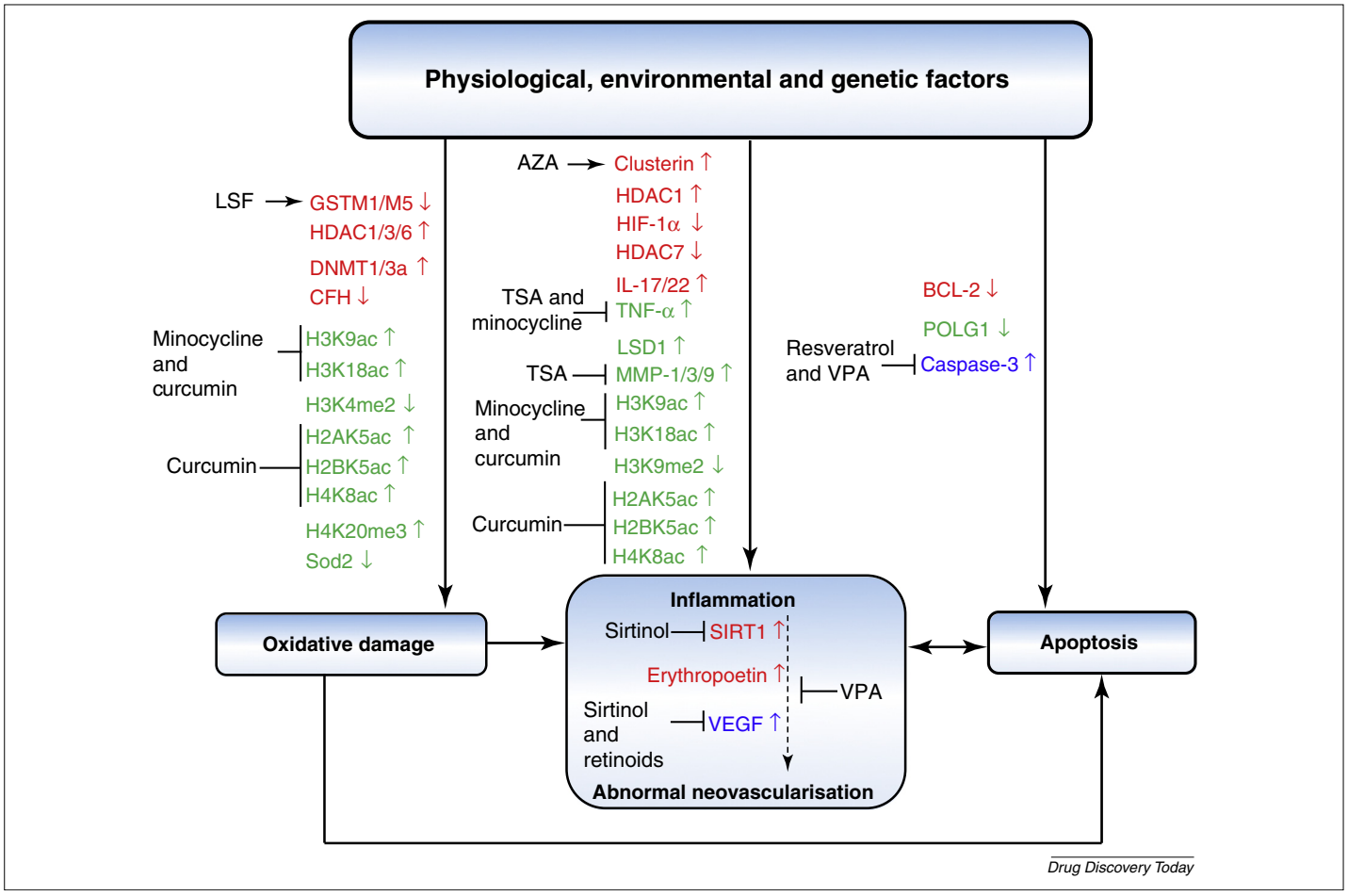

FIGURE 1

Schematic diagram illustrating the role of aberrant epigenetic changes in the modulation of key pathological processes involved and the possible use of chromatin-modifying agents in targeting age-related macular degeneration (AMD) and diabetic retinopathy (DR). The common pathological features of these posterior eye diseases include oxidative damage, inflammation and apoptosis, which can be triggered by physiological (e.g. hyperglycaemia and hyperlipidaemia), environmental (e.g. smoking, ultraviolet radiation and diet) and/or genetic (e.g. familial clustering of disease genes) factors. Inflammation, a tissue response to injury, can be induced by either oxidative damage or apoptosis. Conversely, an excessive inflammatory response can trigger the release of cytokines and leukocyte activation, leading to amplified tissue damage and apoptosis of retinal cells. In these diseases, apoptosis can also be caused by increased oxidative stress via increased release of reactive oxygen species accompanied by a reduced amount of antioxidant. Neovascularisation occurs as part of the healing process following the inflammatory response. However, these newly formed blood vessels are leaky, resulting in retinal cell damage. Epigenetic changes have been reported to modulate the above-mentioned pathological processes and compounds that have chromatin-modifying effects could have the potential to reverse these aberrant epigenetic alterations. The epigenetic changes highlighted in red, green and blue correspond to AMD, DR, and both diseases, respectively. Abbreviations: AZA, 5-aza-2′-deoxycytidine; BCL-2, B cell lymphoma 2; CFH, complement factor H; DNMT1/3a, DNA methyltransferase 1/3a; GSTM1/M5, glutathione S-transferase Mu-1/Mu-5; H2AK5ac, histone H2A acetyl lysine 5; H2BK5ac, histone H2B acetyl lysine 5; H3K18ac, histone H3 acetyl Lysine 18; H3K4me2, histone H3 dimethyl Lysine 4; H3K9ac, histone H3 acetyl Lysine 9; H3K9me2, Histone H3 dimethyl Lysine 9; H4K20me3, histone H4 trimethyl lysine 20; H4K8ac, histone H4 acetyl lysine 8; HDAC1/3/6/7, histone deacetylase 1/3/6/7; HIF-1α, hypoxia-inducing factor 1 alpha; IL-17/22, interleukin 17/22; LSD1, lysine-specific demethylase 1; LSF, L-sulforaphane; MMP-1/3/9, matrix metalloproteinase 1/3/9; POLG1, DNA polymerase gamma 1; SIRT1, sirtuin 1; Sod2, superoxide dismutase 2; TNF-α, tumour necrosis factor alpha; TSA, trichostatin A; VEGF, vascular endothelial growth factor; VPA, valproic acid.

retinal and optic nerve damage in rats with retinal ischaemia following subcutaneous administration of VPA. The authors also reported a corresponding increase in heat-shock protein 70 (Hsp70) transcriptional activity and a significant reduction in the level of caspase 3 activity in the retina of rats treated with VPA, suggesting that ischaemia-induced retinal cell apoptosis is ameliorated by treatment with VPA [57]. Despite these neuroprotective effects, VPA has been reported to affect colour vision discrimination in patients with epilepsy [58]. In addition, VPA was also found to reduce visual acuity and fields in patients with retinitis pigmentosa and to have adverse effects, such as lethargy, stomach irritation and weight gain [59,60]. These important findings necessitate further pharmacological and clinical evaluation before its intraocular use in retinopathies.

Other potential epigenetic-based therapies

Minocycline, a second-generation tetracycline, has been shown to inhibit the expression of pro-inflammatory cytokines and prevent apoptosis of retinal cells in *in vivo* and *in vitro* diabetic models [50]. Kadiyala *et al*. demonstrated a significant decrease in hyperglycaemia-induced acetylation of retinal histones H2, H3 and H4 in diabetic rats treated with minocycline over the duration of the study [50]. A significant reduction in hyperglycaemia-induced acetylation of H3K18 and H3K9 in high glucose-cultured retinal Müller cells treated with minocycline has also been reported. The altered H3K18 and H3K9 acetylation status resulted in a reduction in the expression of tumour necrosis factor (TNF)-α, monocyte chemoattractant protein-1 and glial fibrillary acidic protein [61].

Curcumin, extracted from turmeric, has been investigated in the management of DR owing to its antioxidant and anti-inflammatory properties [62,63]. It has been shown to inhibit p300- and CREB-binding protein (CBP)-HATS, which are transcriptional coactivators of cell growth, differentiation and apoptosis [62]. Oral administration of curcumin was found to inhibit the hyperglycaemia-induced increase in the acetylation of H3K9, H3K18, H4K8, H2AK5 and H2BK5 in the retinas of diabetic rats [64]. However, the authors reported no effect on retinal histone acetylation with intraperitoneal administration of curcumin [64]. These discrepant observations might result from the extensive excretion of curcumin in bile and subsequent transformation to tetrahydrocurcumin and hexahydrocurcumin [65]. Another naturally occurring compound, resveratrol, has also been shown to reduce the glycated haemoglobin A1c level in diabetic rats with 4-month supplementation [66].

The experimental methodology used to investigate the above-mentioned chromatin-modifying agents in this disease are summarised in Table 1 for comparative purposes.

Concluding remarks

Recently, treatment strategies for various diseases (e.g. haematological malignancies) have revolved around targeting the underlying pathological processes at the epigenomic level [29]. These are directed at reversing aberrant chromatin remodelling that dictates the expression of genes involved in regulating cellular processes, such as proliferation, apoptosis, ageing, inflammation and angiogenesis, via the activity of chromatin-remodelling enzymes [8,28,35,67]. The recent discovery of epigenetic modifications in patients with posterior eye diseases has led to interest in the potential use of agents that target these enzymes [8,16,26,48]. However, the research on the use of HDACI, DNMTIs and other potential epigenetic-based therapies in AMD and DR management is relatively young and *in vitro* and *in vivo* studies are ongoing. These results, together with the prudent evaluation of their mechanism of action, specificity and adverse effects, are essential for the advancement of these epigenetic modulators into future clinical trials. The role of aberrant epigenetic changes in the modulation of key pathological processes involved and the possible use of chromatin-modifying agents in targeting posterior eye diseases are summarised in Fig. 1.

Given the complex nature of AMD and DR, it is crucial to establish a database of aberrant methylation and acetylation patterns involved, single nucleotide polymorphisms and genes that are dysregulated in normal eye development and ageing that might predispose one to the disease(s). These anomalies can be detected using high-throughput screening procedures and by GWAS (e.g. Human Epigenome Project). Such a database will help identify epigenetic markers of disease risk and progression, predict drug response and/or adverse effects, facilitate the design of improved epigenetic-based agents and optimise the use of the current agents in the management of these disorders. Furthermore, GWAS are warranted in clinical trials to determine the global epigenetic status following the treatment of patients with AMD or DR with chromatin-modifying agents. Ultimately, such approaches will enable clinicians to devise individualised therapies with optimum long-term therapeutic outcomes and minimal adverse effects, and promise patients 'healthy ageing'.

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