ELSEVIER

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)

1359-6446/06/$ - see front matter http://dx.doi.org/10.1016/j.drudis.2014.03.026

Please cite this article in press as: F.A.A. Kwa, Epigenetic modifications as potential therapeutic targets in age-related macular degeneration and diabetic retinopathy, Drug Discov Today (2014), http://dx.doi.org/10.1016/j.drudis.2014.03.026

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,

Please cite this article in press as: F.A.A. Kwa, Epigenetic modifications as potential therapeutic targets in age-related macular degeneration and diabetic retinopathy, Drug Discov Today (2014), http://dx.doi.org/10.1016/j.drudis.2014.03.026