· 专家述评 ·

Epigenetics—the new era of biomedical and ophthalmological research

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[**Abstract**] Epigenetics is a researching hot topic of worldwide now. Increasing evidence shows that the pathogenesis of human diseases is not only influenced by the abnormalities of genetic factors but also by epigenetic mechanisms. Recent technological advances in epigenomic profiling has led to further understanding of the role epigenetic factors played in development, inflammation, aging, immunity, angiogenesis, tumorigenesis, and stem cell biology. The researchers in ophthalmology should pay close attention to the current research of major epigenetic mechanisms and their involvement in human diseases, especially ocular diseases. Moreover, the potential application of epigenetic drugs in the treatment of common human diseases also should be understood. Finally, the challenges and future perspectives underlying epigenetic research are discussed in this editorial paper.

[**Key words**] Epigenetics; Ocular disease; DNA methylation; Histone modification; Non-coding RNA

表观遗传学：生物医学研究和眼科研究的新时代　Shikun He　Sha Ouyang

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【摘要】 表观遗传学研究已成为生物医学领域的研究热点。越来越多的关于人类疾病发生机制的研究表明，疾病的发生不仅受到遗传因素的影响，也受环境因素的影响，因此表观遗传学因素在各种疾病的发生和发展中发挥很大的作用，眼科疾病的发生也与表观遗传因素有关。迄今为止在表观基因组研究上所取得的进展进一步明确了表观遗传因子在生物发育、炎症、衰老、免疫、新生血管形成、肿瘤以及干细胞生物学等许多复杂的病理生理过程中所扮演的角色。眼科医师应该深入研究表观遗传学三大机制的研究进展及其在各类疾病，尤其是眼科疾病中起到的作用，跟踪近年来应用表观遗传学方法和手段在眼病治疗中取得的进展，明确目前表观遗传学研究发展中面临的主要挑战，关注表观遗传学在眼科研究领域的研究前景。

【关键词】 表观遗传学；眼科疾病；DNA 甲基化；组蛋白修饰；非编码 RNA

With the inspirationof genome-wide association studies (GWAS) and epigenome-wide association studies, the field of epigenetics has garnered increasing attention over recent years. Since the 1990s, with the expansion of epigenetic research, several new concepts and terms such as the epigenome, epigenetic epidemiology, epigenetic pathology, epigenetic disease, epimutation, and epigenetic therapy have been created. Therefore, the importance of epigenetic research to human health has coined the term "the epigenetic era."

1　The major epigenetic mechanisms in biomedical research

Major epigenetic factors include DNA methylation, histone modifications and noncoding RNA (ncRNA).

1.1　DNA methylation

DNA methylation typically occurs in a CpG dinucleotide context. CpG islands are regions with high frequency of CpG sites often at or near transcription start sites of genes. Lower CpG density regions are termed CpG "island

shores". The CpG dinucleotide is the most important site of DNA methylation. CpG methylation silences genes while demethylation activates them. However, recent studies showed that the functional effects of DNA methylation could vary according to the genomic context. The importance of DNA methylation has been further realized. The specific changes in DNA methylation is the most accuracy biomarker for aging research, and the changes of DNA methylation in the blood may suggest the risk of cancer and mortality.

1.2 Histone modifications

Histones are the primary protein components of chromatin, which wrap around DNA to form the fundamental structural units of chromatin called nucleosomes. There are several ways by which histones can be modified: acetylation, methylation, phosphorylation and other modifications such asubiquitination, sumoylation, deamination, β-N-acetylglucosamine, ADP ribosylation, histone tail clipping and histone proline isomerization. Those modifications can either affect the overall chromatin structure or influence the binding of effector molecules, thus playing a significant role in regulating various physiologic and pathological phenomena. In most species, histone H3 is primarily acetylated at lysines 9, 14, 18, 23, and 56, methylated at arginine 2 and lysines 4, 9, 27, 36, and 79, and phosphorylated at ser10, ser28, Thr3, and Thr11. Histone H4 is primarily acetylated at lysines 5, 8, 12 and 16, methylated at arginine 3 and lysine 20, and phosphorylated at serine 1. So far, histone modifications are reported to participate in the mechanism of many processes, including inflammation, neurogenesis and neurodegenerative disease, tumorigenesis, retinal development and chronic stress.

1.3 ncRNA

A ncRNA is a functional RNA molecule that is not translated into a protein. ncRNA genes include highly abundant and functionally important RNAs such as transfer RNA (tRNA) and ribosomal RNA (rRNA), as well as RNAs such as miRNA, siRNAs, piRNAs, and long noncoding RNAs (lncRNAs). Although ncRNAs are not translated into proteins, increasingly evidence showed that many of these RNAs are involved in regulating gene expression. Indeed, ncRNAs are associated with many cellular processes such as development, proliferation, apoptosis, tissue morphogenesis, and tumor growth. miRNAs are the most studied ncRNAs. Genes encoding primary miRNAs are scattered throughout the genomes of eukaryotes. miRNAs may contain target sequences and can undergo cleavage and have been implicated in the regulation of growth and development, cell differentiation and diseases by modification of chromatin structure, and regulation of transcription. Long non-coding RNAs are reported to be associated with many physiological and pathological processes such as development, apoptosis, fibrogenesis, adipogenesis and ischemia.

2 Epigenetics and ocular diseases

The pathogenesis of numerous ocular diseases is believed to be regulated by the interaction of a great diversity of genetic, environmental and epigenetic factors. Extensive studies over recent decades have demonstrated that epigenetic modificationsare involved in various ocular diseases. Focusing on the epigenetic research in eye may provide a better understanding of the pathogenic mechanism of complicated eye disease, which may in turn provide new ideas to find out better treatments of the disease.

2.1 Keratitis

It is widely demonstrated that epigenetic mechanisms are involved in the regulating of inflammatory gene expression, which is activated by the interaction between pathogens and immune cells, suggesting the possibly important role of epigenetic factors in the pathogenesis of keratitis. There are no reports on the role of epigenetic factors such as DNA methylation and histone acetylation in the pathogenesis of fungal keratitis. However, Toll-like receptors (TLRs) can be activated by fungal metabolic products and then causes the alteration of histone acetylation/deacetylation which is similar to that of bacterial infection[1], and then activate downstream nuclear factor-κB (NF-κB) signaling, leading to the production of inflammatory factors that promote the development of fungal keratitis. This implies that the disequilibrium between histone acetylation and deacetylation may be a potentially important mechanism in the pathogenesis of keratitis. Notably, the expression of miRNA-155 was reported to be increased in both herpetic stromal keratitis and bacterial keratitis[2-3]. Herpetic keratitis is a common and recurrent infectious corneal disease. The establishment of latent infection can lead to

recurrence of type1 herpes simplex virus (HSV1) in the cornea. However, at present, the pathogenesis of the latent infection is remained unclear. Recent research shows that the pathogenesis of HSV infection and recurrence is also tightly regulated by epigenetic factors such as DNA methylation and histone acetylation status. Interestingly, HSV1 latent infection can be activated by the application of the histone deacetylase (HDAC) inhibitor trichostatin A (TSA)[4]; the reactivated virus could then be killed using specific anti-HSV treatment, suggesting that epigenetic therapy is a promising new approach in the treatment of latent HSV infection.

2.2 Glaucoma

The pathogenesis of glaucoma is still under investigation. Recent research showed that epigenetic mechanism might contribute to the pathogenesis of glaucoma. It is demonstrated that an increase of global DNA methylation is seen in human glaucomatous lamina cribrosa cells and trabecular meshwork cells compared to normal human[5-6]. Interestingly, hypoxia can induce this global DNA methylation in normal trabecular meshwork cells. Furthermore, significant differences in genomic DNA methylation have been found in peripheral mononuclear cells from patients with open angle glaucoma compared with healthy controls[7].

Besides DNA methylation, histone modification also involve in the pathogenesis of glaucoma. Studies showed that combined HDAC1 and HDAC2 depletion promotes the survival of retinal ganglion cell (RGCs) after injury, and conditional knock out of HDAC3 in RGCs prevent global deacetylation of histone H4 and heterochromatin formation and improving RGCs viability after optic never injury[8-9]. In addition, inhibition of HDAC1 and HDAC3 reduce the loss of RGCs differentiation and preserved the survival of RGCs following optic never injury[10]. These results implicate that HDAC inhibitors may protect vision in patients with optic nerve injuries and might be a promising treatment for glaucoma.

Other epigenetic mechanisms like non-coding RNAs may also play an important role in the pathogenesis of glaucoma. Long non-coding RNA *CDKN2B-AS1* gene, also known as antisense non-coding RNA in the Ink4 locus (ANRIL), can interact with polycomb repressive complex-1 (PRC-1) and PRC-2, leading to transcriptional repression of other genes[11-12]. Furthermore, patients with primary open-angle glaucoma (POAG) carrying alleles of CDKN2B-AS1 single nucleotide polymorphisms (SNPs) are reported to have larger cup-to-disc ratio and lower intraocular pressure and are more likely to develop normal-tension glaucoma and advanced glaucoma[13]. A recent study demonstrated genetic association at *CDKN2B/CDKN2B-AS1* gene contributes to sex bias in normal-tension glaucoma[14].

2.3 Cataracts

The formation and development of cataract is the result of interactions among numerous genetic, environmental, and metabolic factors. Oxidative stress and DNA damage facilitate the pathogenesis of age-related cataract. DNA methylation of DNA repair genes may be involved in the development of cataract. Additionally, a recent study found that hypermethylation of aCpG island of 8-oxoguanine DNA glycosylase 1 (OGG1), a gene coding the OGG1, which is a base excision repair protein involved in DNA repair, leads to reduction of the expression of OGG1, thus contributing to the pathogenesis of age-related cataract[15]. Another DNA repair related gene, *ERCC6*, is also reported to be down regulated through coordinated DNA hypermethylation and histone deacetylation in lens epithelium cells (LECs) of age-related nuclear cataract[16]. Higher DNA methyltransferase (DNMT) and HDAC activity was recently demonstrated to be associated with the onset and formation of age-related nuclear cataract[17]. Notably, a higher expression of 5 DNA methylation-and transcriptional repression-associated genes (*DNMT3B*, *HDAC1*, *HDAC4*, *HDAC9*, and *MBD3*) in LECs of age-related cataract[18]. Methylation binding protein 2 (MeCP2) may play an important role in transforming growth factor (TGF)-β-induced posterior capsular opacification (PCO) after cataract surgery. Importantly, the use of the DNA methylation inhibitor zebularine can inhibit lens epithelial-mesenchymal transformation (EMT) *in vitro*. This result suggests that aberrant DNA methylation may be relevant to PCO. Taken together, those studies support that DNA methylation and histone modification might contribute to the development of cataract.

2.4 Uveitis

In China, the most common types of uveitis are Behçet´s

disease (BD), Vogt-Koyanagi-Harada syndrome (VKH) and acute panuveitis. Although the pathogenesis of uveitis still remains unclear, increasing evidences suggested that epigenetic factors play an important role in the development of uveitis. In the first epigenome-wide study of BD, Hughes et al[19] found that the methylated CpG sites in monocytes and $CD4^+$ cells were significantly different between BD patients and controls. Interestingly, these methylations of the CpG sites can be reversed by DNA methylation inhibitor. These data suggest that aberrant DNA methylation involves in the pathogenesis of BD.

Several microRNAs have been recognized to participant in the pathogenesis of various kinds of uveitis, including BD, experimental autoimmuneuveoretinitis (EAU) and VKH, possibly by regulating many different inflammatory pathways. Evidence has disclosed that NF-κB is involved in the induction of inflammatory response in uveitis[20-21]. In experimental autoimmune anterior uveitis (EAAU), the expressions of miR-146a-5p, miR-155-5p, miR-223-3p, and miR-147b are decreased while the expressions of miR-182-5p, miR-183-5p, and miR-9-3p are elevated[22]. Meanwhile, overexpression of cytokine γ-interferon (IFN-γ), interleukin (IL)-17, IL-12A, IL-1β, and IL-6 and down regulation of IL-10 is observed. A similar experiment showed the upregulation of miR-223 and miR-146a are accordant with the clinical score of the EAU and elevation of IL-1β/ monocyte chemoattractant protein-1 (MCP-1) in the eye with EAU[23]. In addition, 67 miRNAs are differentially expressed between EAU rats versus normal ones, in which 31 miRNAs are downregulated and 36 miRNAs are upregulated[24]. Importantly, the association of microRNA-146a and microRNA-155 and the susceptibility to uveitis is revealed[25-28].

2.5 Age-related macular degeneration

The pathogenesis ofage-related macular degeneration (AMD) is still under investigation. Recently, epigenetic mechanisms including DNA methylation, histone modification and ncRNA have been implicated in the development of AMD. Two differentially methylated CpGs in the promoter of *ARMS2* gene were identified, a top-ranked GWAS locus associated with choroidal neovascularization (CNV) in the blood of CNV patients, and one of the aberrant methylated CpG sites significantly correlated with the risk polymorphism rs10490924, indicating the association of aberrant DNA methylation and susceptibility to CNV[29]. At the same time, differences in DNA methylation in the *PRSS50* gene promoter is observed in both blood and retina between AMD patients and the controls.

In wet AMD, upregulation ofhypoxia inducible factor 1 (HIF1) is associated with increased vascular endothelial growth factor (VEGF) expression. Epigenetic regulation of HIF-1α has been evaluated in cell culture and cancer models. Increased HIF-1α leads to the expression of VEGF, however, the signaling can be inhibited via HDAC1 by upregulating p53 and the Von Hippel-Lindau protein[30]. It is also shown that HDAC7 is able to increase HIF-1α transactivation ability[31]. Notably, VEGF induces HDAC7 nuclear translocation thereby activating proangiogenic gene expression[32]. In addition, hypomethylation of the IL-17 receptor C (IL17RC) promoter has been identified in peripheral blood cells from patients with AMD and is associated with increased expression of IL17RC in their peripheral blood and affect retina and choroid, suggesting that epigenetic regulation of IL17RC may play a role in the pathogenesis of AMD. In contrast, another research group finds no significant difference of methylation level of CpG rich region within the promoter of *IL17RC* in peripheral blood leukocytes from AMD patients and unaffected controls between AMD and controls subjects[33]. These studies suggest that further research should be carried out to have a better understanding of the role of DNA methylation in the pathogenesis of AMD.

MicroRNAs are also involved in the pathogenesis of AMD. The expression of miRNA-184 is recently reported to be inhibited in the retinal pigment epithelium (RPE) of AMD specimen. Using non-biased microRNA arrays and individual TaqMan qPCRs, it is found that in the vitreous humor and plasma of patients with CNV, the expression of miR-146a is increased, while the expression of miR-106b and miR-152 are decreased. The profile of microRNA expression in serum may serve as a non-invasive biomarker for the distinguishing of dry and wet AMD. The higher level of miR661 and miR3121 is observed in serum of patients with dry AMD, however, the increased expression of miR4258, miR889, and Let7 in serum are only seen in patients with wet AMD[34].

2.6 Diabetic retinopathy

Complex intercrossed pathophysiological mechanisms, including genetic and epigenetic factors, take part in the development ofdiabetic retinopathy (DR). Genome-wide DNA methylation is found in the type 1 diabetes with proliferative diabetic retinopathy (PDR). 349 CpG sites representing 233 unique genes are identified differentially methylated between PDR patients and controls, among them 276 CpG sites are hypomethylated while other 73 sites are hypermethylated. Moreover, genes involved in retina and eye development, inflammation, diabetic complications and oxidative stress are found to be associated with these differentially methylated genes in the blood from type 1 diabetes patients with PDR. Interestingly, global DNA methylation levels are significantly higher in DR patients with type 2 diabetes compared with those in patients without retinopathy; notably, an increasing trend in global DNA methylation levels is correlated with the progress of retinopathy. In addition, epigenetic modification is associated with dysfunctional retinal mitochondria in DR, including the mitochondrial DNA methylation and the alteration of mitochondria related genes[35-38].

Diabetic patients have a strong association between the polymorphism in the gene that encodes histone methyltransferases, SUV39H2 and retinopathy[39]. Experimental studies have shown an increased role of H4K20 methylation at the promoter and enhancer regions of superoxide dismutase 2 (SOD2), the gene that encodes mitochondrial superoxide dismutase, in its downregulation in diabetes[40-42]. Increased histone methyltransferase Set7 recruitment at the promoter of NF-κB in hyperglycemic milieu is associated with its increased transcription activity[39,43], and increased acetyl H3K9 at the promoter of matrix metalloproteinsase-9 further decreases transcriptional activity of the master regulator nuclear factor 2 related-factor 2 antigen (Nrf2). The mechanism of histone modification in diabetes may include increased oxidative stress and hypoxia, as in diabetes the retina experiences increased oxidative stress, and hyperglycemia-induced superoxide overproduction activates the major pathways in the development of DR.

MicroRNAs, as the most extensive studied non-coding RNA, are also reported to play an active role in the pathogenesis and progression ofDR. Some researchers identified that shMiR-27b and miR-320a are independently associated with incidence and progression of DR, and the variant of miRNA-126 by rs4636297 SNP significantly associate with severe DR compared with mild DR patients or normal subjects. Importantly, the enhanced expression levels of miRNA-155 is correlated to the progressing retinopathy, and miR-18b is able to inhibit VEGF synthesis and retinal endothelial cell proliferation by targeting insulin like growth factor 1/ insulin like growth factor 1 receptor (IGF-1/IGF1R) signaling pathways[44-47].

2.7 Proliferative vitreoretinopathy

The formation of subretinal, epiretinal and intravitreal fibrotic membranes that is characterized by EMT of RPE is identified as a hallmark of PVR. Several cytokines and growth factors, including tumor necrosis factor (TNF), hepatocyte growth factor (HGF), platelet derived growth factor (PDGF), TGF-β and IL-1, were proved to be involved in the pathogenesis of PVR. Recently, MeCP2 has been demonstrated to play a pivotal role in EMT of RPE cells and may be a regulator of the pathogenesis of PVR. Furthermore, 5-AZA-dC, which suppresses the methylation of DNA leading to increased gene expression, inhibits MeCP2 expression and suppresses methylation of RASAL1, a gene of the RAS-GAP family which is known to be decreased in fibrotic tissue. Besides DNA methylation, histone modification is also involved in the pathogenesis of PVR. TSA might suppress the TGF-β-induced EMT of RPE cells through down-regulating the Jagged/Notch signaling pathway. Several non-coding RNAs have a potential role in the pathogenesis of PVR. A microarray performed by Zhou et al[48] identified 78 abnormally expression of lncRNAs in the epiretinal membranes of PVR patients, and MALAT1 expression level is significantly increased in PVR patients and associated with the severity of PVR in TGF-β treated RPE cells; silencing of MALAT1 decreases the TGF-β-induced EMT and proliferation of RPE cells, suggesting a potential therapeutic value of epigenetic treatment in the prevention and treatment of PVR.

2.8 Amblyopia and myopia

Amblyopia and myopia may be subjected to the regulation by epigenetic factors. During the development of deprivation amblyopia, the histone modification plays a potential role. Chronic intraperitoneal administration of the

HDAC inhibitors (valproic acid or sodium butyrate) result in a complete recovery of visual acuity and tested visual evoked potentials after the sutures are removed, suggesting that epigenetic treatment, like histone deacetylation inhibitors, might help to prevent visual loss of amblyopia. Although there is not that much research reported the association between epigenetic mechanism and amblyopia, it is promising epigenetic treatment might provide more options for amblyopia patients.

A recent study in discordant monozygotic twins revealed several environmental risk factors for the development of myopia, which raises the possibility that long-lasting unhealthy environmental exposure may alter the expression of epigenetic factors that might in turn affect ocular growth and the development of myopia[49]. Another report by Zhou et al[50] showed that there is a high frequency of methylation in CpG sites of *COL1A1* promoter during the development of myopia in mice. In addition, a functional SNP of *PAX6* gene is tightly related to extreme myopia, and microRNA-328 may play an important role in the pathogenesis of myopia by regulation of the expression of the *PAX6* gene[51-52].

2.9 Uveal melanoma

Uveal melanoma, with high mortality rate and frequent metastases, is the most common primary ocular tumor in adults. Multiple studies already reported that epigenetic mechanisms are involved in the tumor genesis and metastases. Aberrant DNA methylation of the promoter of a number of genes, including *RASSF1A*, *pINK4*a, *TIMP3*, *RASEF*, $p16^{INK4a}$, *MGMT*, and *EFS*, has been proved to associate with uveal melanoma[53-55]. In addition to DNA methylation, histone modification has also been implicated in the pathogenesis of uveal melanoma. Recently, tenovin-6, a class Ⅲ-specific HDAC inhibitor, is reported to induce apoptosis and suppresses migration of the cells of uveal melanoma[56]. Similarly, class Ⅱ-and class Ⅰ-specific HDAC inhibitors suppress the growth of uveal melanoma[57-58]. Furthermore, other epigenetic factors such as miRNAs are also involved in the pathogenesis of uveal melanoma. Interestingly, the effect of miRNAs on uveal melanoma is variable, miRNA-124a, miRNA-137, miRNA-32, miRNA-144 act as tumor suppressor[59-62], while miRNA-20a and miRNA-454 may function as oncogenes which promote cell growth and migration[63-64]. Notably, lncRNA *PAUPAR* may serve as a marker of uveal melanoma.

2.10 Retinoblastoma

Gene mutation of *RB1*, a prototypic tumor suppressor gene, has long been considered as the most important pathogenesis of retinoblastoma (RB). However, growing evidence showed the significance of epigenetic factors in regulating the tumorigenesis of RB. Studies demonstrated that promoter hypermethylation of *RB1* and other tumor suppressor genes such as $p16^{INK4A}$, *RASSF1A*, *MGMT*, *NEUROG1*, *GSTP1*, *APC*, and *DAPK*, are involved in the development and progression of RB[65-73]. Differently expressed non-coding RNAs may also be involved in the pathogenesis of RB, and miRNA-183, miRNA-204, miRNA-101, miRNA-449a/b, miRNA-31, miRNA-200a, miRNA-34a and miRNA-365b-3p are downregulated in RB and enforced expression of these miRNAs inhibits the proliferation and invasion of human RB cells by targeting different signaling pathways[74-80]. However, miRNAs such as miR-17-92 cluster are demonstrated to be oncogenic miRNAs which promote the proliferation of the cells of RB[81-82]. Recently, two lncRNAs, BRAF-activated noncoding RNA (BANCR) and maternally-expressed gene 3 (*MEG3*) are proved to participant in development of RB. BANCR is overexpressed in RB tissues and cell lines and high levels of BANCR expression is correlated with poor prognosis of RB. Knocking down the expression of BANCR significantly inhibits the RB cell proliferation, migration and invasion. *MEG3*, on the other hand, is demonstrated to be able to inhibit RB cell growth by negatively regulating the Wnt/β-catenin pathway[83]. These data imply the possibility that non-coding RNAs might act as a promising prognostic biomarker and therapeutic target of RB. Moreover, a recent study showed that higher histone deacetylase 9 (HDAC9) expression in RB tissue is correlated with poor prognosis of RB.

3　Epigenetics and pharmacotherapeutics

As discussed above, epigenetic mechanisms are involved in the regulation of many human physiologic functions and pathogenesis of numerous diseases. With its versatile and reversible nature, it is not surprising that epigenetic mechanisms are considered as valuable targets for novel efficient strategies of diagnosis, prevention and treatment. Epigenetic-based therapies include inhibition of DNA

methylation, inhibition of HDAC, restoring the overall epigenetic status of the pathological chromatin, restoring transcriptional dysregulation and beyond epigenetics and transcription. Actually, some epigenetic modifying drugs, such as some DNMT inhibitors and HDAC inhibitors, have already been approved by the US Food and Drug Administration (FDA) for clinical use. Beside DNA methylation inhibitor, DNA methylation can be affected by HDACi 4b (HDAC inhibitor) exposure. Regulation of DNA methylation by DNA methylation inhibitor or histone modification drugs is able to alter the DNA methylation, thereby providing a new concept for the combination of application of epigenetic drugs. Interestingly, the combinations of epigenetic modifying drugs targeting different regulatory components with other traditional therapies may be considered as a promising approach to improve therapeutic efficacy for patients suffering from ocular diseases and other human disease.

However, there are many challenges such as pharmacokinetic properties, stability and specificity issues in developing epigenetic drugs. Most of DNMT inhibitors are unstable in aqueous solutions, and they can be degraded by nucleotide salvage enzyme *in vivo*. The half-life of some epigenetic drugs, such as decitabine and 5-Aza, is pretty short in plasma. However, it is found that the half-life of zebularine, a DNMT inhibitor, is up to 500 hours at certain conditions. Another common phenomenon is the variation of individual in reactions to medications, which is related to epigenetic factors variation, for example, cytochrome P450 is responsible for different responses to the same drug in different individuals, and importantly, the P450 expression is regulated by DNA methylation. Moreover, the effects of epigenetic drugs, such as HADC inhibitors, are reversible after removing the drugs, thus requiring long-term administration of the drugs to achieve a continuous effect on biological targets. More importantly, the major concern about the application of epigenetic drugs is lack of target specificity. The current DNMT inhibitors work in global demethylation; the HDAC inhibitors can effect multiple HDAC isoforms; similarly, one non-coding RNA can act on many related targets. Therefore, the development of higher specific and efficient epigenetic drugs that avoid extensive off-target effects and have pharmacokinetic stability is of great importance.

4　Paying attention to the application of epigenetic study in ophthalmology

The past decades have witnessed great development in epigenetic researches, and the improvements in technology, such as the genome-wide ChIP-Sequencing (ChIP-Seq) technology and some high-resolution genome-wide mapping technologies, make it easier to investigate the underlying regulatory mechanism of epigenetic factors in human physiological and pathological process. Although the whole map of epigenetic modifications remains to be illustrated, it is clear that the alteration of epigenetic factors play a significant role in a variety of human diseases, including numerous ocular diseases. Therefore, more extensive epigenetic research is required to explore the full spectrum of epigenetic modifications as there are still some critical questions need to be addressed, such as what triggers the differentially expression of epigenome in different individuals? How do epigenetic modifications interact with known genetics pathways in the pathogenesis of a certain disease? How do epigenetic markers correspond to different specialized cell types and how do they vary among different diseases and individuals? How do epigenetic factors regulate ocular development, homeostasis and disease? In addition, the effective therapies or preventive and diagnostic strategies will be highlighted by thorough epigenetic investigations. Therefore, the researchers in ophthalmology should be to pay close attention to the association of epigenetics with eye diseases.

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