YALE JOURNAL OF BIOLOGY AND MEDICINE 89 (2016), pp.597-600.

MINI-REVIEW YJBM

Epigenetics and Common Ophthalmic Diseases

Wendy Li, BS; Ji Liu, MD; Jennifer A. Galvin*, MD

Department of Ophthalmology and Visual Science, Yale School of Medicine, New Haven, CT

The study of ocular diseases and epigenetic dysregulation is an emerging area of research. The knowledge from the epigenetic mechanisms of DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs regarding the pathogenesis of ocular diseases will be helpful for improved treatment modalities for our patients. In particular, we focus upon the how epigenetic regulatory mechanisms impact five common ocular diseases: age related macular degeneration, age-related cataract, pterygium, retinoblastoma, and uveal melanoma. Hence, the foundation of this research paves the way for future specific therapeutic targets to treat and prevent vision loss.

INTRODUCTION

What is epigenetics? Epigenetics is the study of heritable changes in the gene expression without any change in the DNA sequence [1]. This includes DNA methylation, histone modification, chromatin remodeling, and the use of non-coding RNAs. The methylation of DNA at the gene promoter modifies DNA accessibility to transcription factors or helps recruit silencing-associated proteins [2]. This abnormal methylation results in gene silencing and hence, gene expression alterations [3]. Histone modifications occur primarily at the N-terminal tails, including methylation, acetylation, sumoylation, and phosphorylation [4]. Chromatin modifiers, such as histone acetyltransferases (HATs†), histone deacetylases (HDACs), and lysine methyltransferases (KMTs), modify histones and other proteins [5]. Chromatin remodeling complexes contain an enzymatic ATPase subunit, such as SWI/SNF or ISWI, and use ATP hydrolysis to allow access to DNA for DNA binding proteins, such as BRG1, Ino80 [6]. Non-coding RNAs include small infrastructural RNAs: nuclear, nucleolar, ribosomal as well as regulatory RNAs: microRNAs, long non-coding RNAs, small-interfering RNAs, and Piwi-interacting RNAs [7]. These non-coding RNAs mediate post-transcriptional downregulation of gene expression [8].

Understanding epigenetic regulatory mechanisms in the pathophysiology processes of ophthalmic disease will help us treat and manage our patients' vision loss. This mini-review will focus on two areas of significant epigenetic research in human cell lines and tissues: DNA methylation and non-coding RNA molecules, in five common ophthalmic diseases. In the past decade, prior studies have identified epigenetic modifications in cancer, neurologic disease, and autoimmune disease [9].

Although the epigenetic findings of chromatin remodeling and modifications of histones have been noted in models of mice, *Drosophila*, and zebrafish, we will focus on the genes involved in abnormal DNA methylation and abnormal miRNA expression as these have been the two most significant areas of epigenetics applied to human ophthalmic disease [10].

AGE RELATED MACULAR DEGENERATION

Age-related macular degeneration (AMD) is the leading cause of irreversible loss of central vision in our patients over the age of 65 years [11]. From 2000 to 2010, the number of AMD patients in the United States increased from 1.75 million to 2.07 million [11]. The degeneration of the retinal pigment epithelium (RPE) layer

*To whom all correspondence should be addressed: Jennifer A. Galvin, MD, Assistant Professor, Department of Ophthalmology and Visual Science, Yale School of Medicine, 40 Temple Street, Suite 3B, New Haven, CT 06510, Tel: 203-785-2020, Fax: 203-785-5909, email: Jennifer.a.galvin@yale.edu.

†Abbreviations: AMD, Age-related Macular Degeneration; RB, Retinoblastoma; HAT, Histone Acetyltransferase; HDAC, Histone Deacetylase; KMT, Lysine Methyltransferase; RPE, Retinal Pigment Epithelium; GST, Glutathione S Transferase.

Keywords: Age-related macular degeneration, cataract, epigenetics, pterygium, retinoblastoma, uveal melanoma

in patients with AMD is a progressive process which causes significant problems with how the retina processes light. The major physiologic roles of the RPE involve visual pigment regeneration, synthesis and remodeling of the interphotoreceptor matrix, transport of nutrients and ions to photoreceptor cells and removal of waste products from photoreceptors, absorption of light via the pigmentation, and adhesion to the retina [12]. Clearly, the RPE is vital for normal visual function and the progressive pathologic disease state of the RPE in patients with AMD has severe consequences. What is known about epigenetics and AMD? Researchers identified 231 genes with altered methylation patterns in the promoter regions in monozygotic AMD twin patients and dizygotic AMD twin patients [13]. In particular, Wei et al. described the DNA hypomethylation in the promoter region of IL17RC, the receptor for IL-17A and IL-17F in the AMD twin patients [13]. In addition, patients with AMD had an increased macular expression of IL 17-RC transcripts and protein as compared to non-AMD controls [13]. This epigenetic research suggests that AMD patients may be more susceptible to IL-17 mediated inflammatory responses, which in turn may contribute to AMD pathogenesis.

Another study examined the genome-wide methylation status in post-mortem retinal pigment epithelium and choroid from AMD patients. Hypermethylation was identified in the promoter regions of two glutathione S transferase isoforms: GSTM1 and GSTM5; this correlated with decreased mRNA and protein levels [14]. Of note, glutathione S transferases (GSTs) are a key part in the defense against oxidative stress; epigenetic downregulation of GSTs could contribute to increased vision loss due to the increased susceptibility to oxidative stress in AMD patients.

CATARACT

Age-related cataract, caused by an opacification or cloudiness of the crystalline lens, is the leading cause of blindness worldwide. Of note, cataract surgery is the removal of the natural crystalline lens with replacement of an intraocular lens implantation. This surgery is the most common outpatient surgery for adults in the United States. According to the National Eye Institute at the National Institute of Health, approximately half of all Americans have had a cataract or cataract surgery by age 80 [15]. What is known about epigenetics and age-related cataracts? Researchers found that for *CRYAA*, the gene for alpha-A-crystallin in human lens epithelial cells, the protein levels were downregulated and the *CRYAA* promoter region was hypermethylated [16]. In the future, DNA methylation may provide a targeted therapy with *CRYAA* to prevent the degree of opacification of the natural crystalline lens in our adult patients with age-related cataracts. Other researchers identified higher levels of miR-34a with a positive correlation with the severity of lens opacity [17]. These research findings suggest a potential role for miRNA in treatment modalities for age-related cataracts.

PTERYGIUM

A pterygium is a triangular or wedge-shaped epithelial fibrovascular growth and proliferation of the conjunctival tissue onto the peripheral cornea, growing over the limbal area. This is a benign condition, but it is exacerbated by exposure to UV light and has an increased prevalence in patients who work outdoors and live in warm and tropical climates. In one of the larger studies, the Barbados Eye Study, researchers examined 2781 patients between 40 to 84 years old. Cases of pterygium were found among 23.4 percent of the 2617 patients identified as black, 23.7 percent of the 97 patients identified as black and white, and 10.2 percent of the 59 patients identified as white [18]. Worldwide, the prevalence studies of pterygium have varied from 3 to 24 percent, depending on the population studied [18-20].

Typically, patients with pterygium do not have central vision loss; however, the abnormal growth and proliferation of cells onto the peripheral cornea can induce astigmatism as well as disturb the tear film, contributing to dry eyes. The histopathology of pterygium shows elastotic degeneration of the stromal collagen with subepithelial fibrovascular tissue [21]. In particular, the destruction of Bowman's layer by the advancing fibrovascular tissue results in a corneal scar [21]. With a peripheral corneal scar, ocular surface dryness is a common clinical finding in pterygium patients. In severe cases, the fibrovascular tissue grows aggressively, involving the central cornea, causing central vision loss. What is known about epigenetics and pterygium? Researchers found that altered methylation patterns in the genes encoding transglutaminase 2 (TGM2), metalloproteinase 2 (MMP2), and CD24 [22]. In particular, hypermethylation at the promoter region of TGM2 indicated decreased transcript and protein levels while hypomethylation of regions of MMP2 and the promoter region of CD24 indicated increased transcript and protein levels [22]. These three genes are also involved in cell adhesion and extracellular matrix remodeling, our further understanding of the pathophysiologic mechanisms and how they relate to epigenetics could improve our treatment modalities of pterygiums.

RETINOBLASTOMA

Retinoblastoma (RB) is the most common primary intraocular tumor in children [23]. This form of cancer is not only severely threatening to vision, but threatening to life, as there may be extension into the optic nerve and/or involvement of the brain. What is known about epigenetics and RB? Researchers have identified hypermethylation in the promoter regions of ten genes: *MSH6*, *CD44*, *PAX5*, *GATA5*, *TP53*, *VHL*, *GSTP1*, *MGMT*, *RB1*, and

CDKN2 [24]. These ten genes are important in a spectrum of cancer-related pathways: DNA repair, tumor suppression, and cell-to-cell interactions [24]. In addition, other researchers found that in 82 percent of their patients with RB, the tumor suppression gene of *RASSF1A* was silenced by DNA methylation [25]. For the children diagnosed with RB, the dysregulation of methylation identified in these eleven genes is a further tool for targeted treatment to improve the prognosis of this ocular cancer [25]. In addition, microarray analysis reveals that several small non-coding RNA molecules are aberrant and are highly expressed in patients with RB: miR-494, let-7e, miR-513-1, miR-513-2, miR-518c, miR-129-1, miR-129-2 [26]. Other researchers identified that miR-34a, miR-17/92, miR-I29-2 functions as a tumor-suppressor in RB cells [27].

UVEAL MELANOMA

Uveal melanoma is an aggressive tumor in adults arising from the pigmented cells of the uvea, including the iris, ciliary body, and choroid. This malignant cancer is a threat to vision as well as a threat to our patients' lives--regarding its growth and potential for metastasis. Uveal malignant melanoma is the most common primary intraocular tumor in all age groups [23]. What is known about epigenetics and uveal melanoma? Researchers identified that the tumor suppressor gene, *RASSF1A*, was hypermethylated in 50 percent of the archived frozen tumor specimens and in 91 percent of the uveal melanoma cell lines [28]. Other researchers found that in 23 uveal melanoma biopsies, the *hTERT* gene showed a higher frequency of hypermethylation, in 52 percent of the biopsies [29]. Furthermore, the *EFS* gene was noted to have bi-allelic methylation in uveal melanoma biopsies, with a poor prognosis for this cohort of patients [30]. Abnormal miRNA expression in uveal melanoma has been observed by researchers with a downregulation of a tumor suppressor and regulator of the transcription factor microphthalmia-associated transcription factor, MITF, in uveal melanocytes: miR-137 [31]. In this way, the loss of miR-137 activity may be a key part of tumorigenesis of uveal melanoma [31].

CONCLUSIONS AND OUTLOOK

Our mini-review of how the study of epigenetics has impacted five common ophthalmic diseases focused upon DNA methylation and non-coding RNAs. With these epigenetic alterations, the progression of AMD, age-related cataracts, and pterygium will be better understood regarding the interactions between gene expression changes and environmental exposures [32].

Epigenetic information is usually different from cell to cell in different developmental stages and this epigenetic targeted-approach for therapeutic markers would potentially lower toxicity and side effects for patients. Regarding the prior research of epigenetic targeted therapy for cancer, the future treatment of ocular cancers, such as RB or uveal melanoma, may benefit from the development of an epigenetic map. Recently, clinical trials of epigenetic therapeutics in ophthalmic disease are focused on small RNA-based therapeutics for the retina, and in particular, AMD. In the future, our conversations with our patients will emphasize that specific therapeutics to epigenetic alterations will be a more effective treatment modality to provide hope to prevent irreversible vision loss.

REFERENCES

1. Holliday R. Epigenetics: A Historical Overview. Epigenetics. 2006;(1):76-80.
2. Franchini DM, Schmitz KM, Petersen-Mahrt SK. 5-Methylcytosine DNA Methylation: More than losing a methyl group. Annu Rev Genet. 2012;(46):419-441.
3. Costello JF, Fruhwald MC, Smiraglia DJ, Rush LJ et al. Aberrant CpG-island methylation has non-random and tumour-type-specific patterns. Nat Genet. 2000;24(2):132-138.
4. Suganuma T, Workman JL. Signals and combinatorial functions of histone modifications. Annu Rev Biochem. 2011;(80):473-499.
5. Kouzarides T. Chromatin modifications and their function. Cell. 2007;128(4):693-705.
6. Alabert C and Groth A. Chromatin replication and epigenome maintenance. Nat Rev Mol Cell Biol. 2010;(13):153-167.
7. Estellar M. Non-coding RNAs in human disease. Nat Rev Genet. 2011;(12):861-74.
8. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136(2):215-233.
9. Portela A, Estellar M. Epigenetic modifications and human disease. Nat Biotechnol. 2010;28(10):1057-1068.
10. Liu MM, Chan CC, Tuo J. Epigenetics in Ocular Diseases. Current Genomics. 2013;14(3):166-172.
11. Klein R, Chon CF, Klein BE et al. Prevalence of Age-Related Macular Degeneration in United States Population. Arch Ophthalmol. 2011;129(1):75-80.
12. Marmor MF, Wolfensberger TJ eds. The Retinal Pigment Epithelium: Function and Disease. New York: Oxford Press, 1998. p103-134.
13. Wei L, Liu B, Tuo J, Shen D et al. Hypomethylation of the IL17RC Promoter Associates with Age-Related Macular Degeneration. Cell Rep. 2012;(2):1151-1158.
14. Hunter A, Spechler PA, Cwanger A, Song Y et al. DNA Methylation is associated with altered gene expression in AMD. Invest Ophthalmol Vis Sci. 2012;53(4):2089-2105.
15. The National Eye Institute (NEI) is a part of the National Institute of Health (NIH) and the federal government's lead agency for vision research. Available September 2015 at http://www.nei.nih.gov.
16. Zhou P, Luo Y, Liu X, Fan L et al. Down-regulation and CpG island hypermethylation of CRYAA in age-related nuclear cataract. FASEB J. 2012;26:4897-4902.
17. Chien K, Chen S, Liu J, Chang H et al. Correlation between microRNA-34a levels and lens opacity severity in age-related cataracts. Eye (Lond). 2013;27:883-888.
18. Luthra R, Nemesure BB, Wu SY et al. Frequency and Risk Factors for pterygium in the Barbados Eye Study. Arch Ophthalmol. 2001;119(12):1827-1832.
19. Cajucom-Uy H, Tong L, Wong TY et al. The prevelance and risk factors for pterygium in urban Malay population: Singapor Malay Eye Study (SiMES). BJO. 2010;94(8):977-981.
20. Ma K, Xu L, Jie Y et al. Prevalence of and factors associated with pterygium in adult Chinese: the Beijing Eye Study. Cornea. 2007;26(10):1184-1186.

21. Eagle RC Jr. Eye Pathology: An Atlas and Text. 2nd Ed. New York: Wolters Kluwer, 2011. p85
22. Riau AK, Wong TT, Lan W, Finger SN et al. Aberrant DNA Methylation of matrix remodeling and cell adhesion related genes in pterygium. PLoS One. 2011;6(2):e14687.
23. Shields JA and Shields CL. Intraocular Tumors: An Atlas and Textbook. 2nd Ed. New York: Wolters Kluwer, 2008. p294; p85.
24. Livide G, Epistolato MC, Amenduni M, Disciglio V et al. Epigenetic and copy number variation analysis in retinoblastoma by MS-MLPA. Path Oncol Res. 2012;18(3):703-712.
25. Choy KW, Lee TC, Cheung KF, Fan DS et al. Clinical implications of promoter hypermethylation in RASSF1A and MGMT in retinoblastoma. Neoplasia. 2005;7:200-206.
26. Zhao JJ, Yang J, Lin J, Yao N et al. Identification of miRNAs associated with tumorigenesis of retinoblastoma by miRNA microarray analysis. Childs Nerv Syst. 2009;25:13-20.
27. Wang J, Wang X, Wu G, Hou D et al. MiR-365b-3p, down-regulated in retinoblastoma, regulates cell cycle progression and apoptosis of human retinoblastoma cells by targeting PAX6. FEBS Lett. 2013;587:1779-1786.
28. Maat W, van der Velden PA, Out-Luiting C, Plug M et al. Epigenetic inactivation of RASSF1a in uveal melanoma. Invest Ophthalmol Vis Sci. 2007;(48):486-490.
29. Moulin AP, Clement G, Bosman FT, Zografos L et al. Methylation of CpG island promoters in uveal melanoma. Brit J Ophthalmol. 2008;92:281-285.
30. Neumann LC, Weinhausel A, Thomas S, Horsthemke B et al. EFS shows biallelic methylation in uveal melanoma with poor prognosis as well as tissue-specific methylation. BMC Cancer. 2011;11:380.
31. Chen X, Wang J, Shen H, Lu J et al. Epigenetics, microRNAs, and carcinogenesis: functional role of microRNA-137 in uveal melanoma. Invest Ophthalmol Vis Sci. 2011;52(3):1193-1199.
32. Yan B, Yao J, Tao ZF, Jiang Q. Epigenetics and Ocular Diseases: From Basic Biology to Clinical Study. J Cell Physiol. 2014;229:825-833.