HHS Public Access

Author manuscript

Ocul Surf. Author manuscript; available in PMC 2020 July 03.

Published in final edited form as:

Ocul Surf. 2020 July ; 18(3): 383–395. doi:10.1016/j.jtos.2020.04.001.

Epigenetic regulation of anterior segment diseases and potential therapeutics

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Abstract

In recent years, technological advances in sequencing have accelerated our understanding of epigenetics in ocular development and ophthalmic diseases. We now know that epigenetic modifications are necessary for normal ocular development and biological processes such as corneal wound healing and ocular surface repair, while aberrant epigenetic regulation underlies the pathogenesis of a wide range of ocular diseases, including cataracts and various diseases of the ocular surface. As the epigenetics of the eye is a constantly changing field of medicine, this comprehensive review focuses on innovations and scientific discoveries related to epigenetic control of anterior segment diseases that were published in the English literature in the past five years. These recent studies attempt to elucidate therapeutic targets for the anterior segment pathological processes. Already, recent studies have shown therapeutic potential in targeting epigenetic mechanisms of ocular disease, and new epigenetic therapies are on the verge of being introduced to clinical practice. New drug targets can potentially emerge as we make further discoveries within this field.

Keywords

Epigenetics; anterior segment; ophthalmic therapeutics

1. Introduction

The field of epigenetics encompasses the reversible, molecular modifications that regulate gene expression, without changing the original DNA sequence. As sequencing technology advances, recent studies have elucidated the role of epigenetics in aging and also in a variety of complex, multifactorial diseases within different fields of medicine including cancer, diabetes, autoimmune, vascular and neurodegenerative diseases. Epigenetic factors may help to explain the variable onset and severity of these diseases and also to serve as biomarkers and inspire targeted pharmaceutical development[1]. Several epigenetic mechanisms can interact with genetic factors to change disease phenotypes. For example, dysregulation of the chromatin state and mutations in chromatin remodeling genes have been found in various

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Conflict of Interest Statement: There is no conflict of interest to declare.

diseases; DNA methylation patterns, which have an important positive and negative role in the regulation of transcription, have also been found to regulate cancer progression[2–5]; post-translational histone modifications, which changes patterns of transcription further modulate disease processes including cardiovascular and neurodegenerative disease[6,7]. In addition, various non-coding RNA have been shown to regulate and inhibit genes in a variety of disease. These mechanisms are often influenced by environmental factors, such as lifestyle, diet, and exposure to toxins[1].

Only recently have epigenetic advances been made in the study of ocular diseases. DNA methylation, chromatin remodeling and various non-coding RNAs have been established as essential mediators in normal ocular development[8–15]. Many believe that these epigenetic mechanisms could be the missing link to understanding disease pathogenesis[1,5–8,16]. Numerous ophthalmic diseases are not fully explained by discrete genetic mutations, suggesting epigenetics may play a role; meanwhile, identical twin studies have shown that the heritability of ophthalmic diseases varies from 27% for diabetic retinopathy[17] to 90% for myopia[18]. Epigenetic changes resulting from environmental factors may play a significant role, and interventions to modify these factors hold increasing promise[1,6,19,20]. Research in epigenetics can further our understanding of ocular disease pathogenesis and revolutionize novel epigenomic-targeted treatments of ocular pathologies.

Although reviews on ocular epigenetics have explored various posterior segment diseases including diabetic retinopathy and age-related macular degeneration, there is a paucity of comprehensive review focused exclusively on the anterior segment diseases and its therapeutic potential in anterior segment diseases. In addition, this field of medicine is constantly changing, and emerging new therapeutic options are becoming more available in the clinics on a yearly basis. This article aims to review recent developments pertaining to epigenetics of the anterior segment and its implications in future research and therapeutic potential.

2. Search strategy

Both primary and secondary literature searches were performed using PubMed. The initial step was a primary literature search in PubMed for original research articles in English published within the last 5 years (January 2014-April 2019). This step focused on recently discovered pathways, associations, and therapeutic targets that highlight the relevance of epigenetics on the anterior segment of the eye. This literature search was organized sequentially by mechanism of epigenetic regulation, i.e. DNA methylation, post-translational histone modification, ATP-dependent chromatin remodeling, and non-coding RNA regulation. Characteristics of these studies, e.g. animal model, tissue type, results, significance, were collected.

Our detailed PubMed search for relevant studies on DNA methylation in the anterior eye segment used the terms (“DNA methylation” OR “DNMT” OR “methyltransferase”) AND (“ocular development” OR “cornea” OR “lens” OR “uvea” OR “conjunctiva” OR “trabecular meshwork”) AND English [Language] AND ((“2014/01/01” [PDAT]: “2019/04/01” [PDAT])). Subsequent searches used the same terms, except the first search

term (“DNA methylation” OR “DNMT” OR “methyltransferase”) as follows: for studies on post-translational histone modification, we used the terms (“post-translational histone”) AND (“modification” OR “acetylation” OR “deacetylation” OR “methylation” OR “phosphorylation” OR “ubiquitination” OR “HDACi”). For studies on ATP-dependent chromatin remodeling, we used the terms (“chromatin remodeling” OR “SWI” OR “SNK” OR “ISWI” OR “BRM” OR “ATP nucleosome”). For non-coding RNA regulation, we used the terms (“non-coding RNA” OR “RNA interference” OR “RNAi” OR “microRNA” OR “miRNA” OR “SiRNA”). In addition, a detailed search of PubMed was conducted using the terms “epigenetics” AND (“anterior eye” OR “cornea” OR “lens” OR “trabecular meshwork” OR “uvea” OR “ocular development”) to manually identify relevant articles that were missed in the above searches.

This was followed by a secondary literature search that manually examined the references used by articles identified in the primary literature search. In particular, this search focused on earlier original research studies that helped lay the foundation for more recent research studies. This secondary literature search did not have date constraints and included articles published since 1991 (the earliest year of publication for the seminal papers cited by literature in our primary search[10]), in order to provide adequate context for more recent studies, which were the focus of this review. A manual literature search was also conducted on the definitions and mechanisms regarding the epigenetic mechanisms studied.

3. DNA methylation and its role in anterior segment diseases

DNA methylation, perhaps the most widely investigated epigenetic mechanism, plays a critical role in development by facilitating transcriptional silencing, X-chromosome inactivation, and genomic imprinting. Methylation of DNA is carried out by enzymes called DNA methyltransferases (DNMTs), each with distinct functions. DNMT1 maintains and restores methylation patterns during DNA replication, whereas DNMT3a/3b set up *de novo* methylation patterns, primarily during embryonic development. In humans, the 5’ cytosine is methylated to produce 5-methylcytosine, which fundamentally results in gene silencing, but also results in genomic imprinting and X-chromosome inactivation during embryonic development[1,21].

Our understanding of DNA methylation in the eye is advancing rapidly. Bonnin et al. (2014) created the first index of DNMT expression profiles in the human eye, demonstrating tissue-specific expression patterns of DNMT transcripts found in the cornea, conjunctiva, anterior lens capsule, and trabecular meshwork[9].

3.1. DNA methylation and corneal wound healing

DNMTs are highly expressed in the cornea[9]. Recently, it was found that DNMTs play a role during corneal epithelial wound healing. In a mouse model, Luo et al. (2019) found that global DNA hypermethylation occurs during corneal epithelial wound healing in vitro due to increased expression of DNMT1 and DNMT3B[22]. These subfamilies of DNMTs appear to contribute to the control of epithelial cell migration, differentiation and proliferation. In addition, inhibition of DNMT1 expression appears to slow down corneal epithelial healing

in mice. Therefore, they conclude that upregulating DNMT1 methylation activity is a potential therapeutic strategy to facilitate corneal epithelial wound healing.[22]

3.2. DNA methylation and Fuchs Dystrophy

In addition to corneal epithelial wound healing, DNA methylation was recently found to play a role in metabolism, fluid transport functions, and structural organization in corneal endothelial cells[16]. Khuc et al. (2017) investigated DNA methylation changes in Fuchs endothelial corneal dystrophy (FECD), particularly in the late-onset subtype which is not classically associated with genetic mutations[23,24]. Thus, the authors sought to find the role of epigenetics in FECD. In this study, DNA methylation patterns in endothelium of patients with FECD were compared to normal human endothelial tissue. It was found that hypermethylated genes were associated with defects in fluid transport and metabolism, while hypomethylated genes were associated with activation of cytoskeletal organization, which allows the corneal endothelium to function as a physical barrier that prevents excess fluid from entering the corneal stroma[16]. Thus, the authors conclude that altered DNA methylation patterns may play a role in corneal edema and subsequent loss of corneal transparency in FECD, and may serve as a novel therapeutic target.

3.3. DNA methylation and development of the lens and formation of cataracts

The integral role of DNA methyltransferases in lens development has been well established, with an early study (Peek et al., 1991) showing the association of de-methylation of the γ-crystallin promoter gene with the physiologic differentiation of lens epithelial cells in a rat model. These DNA methylation patterns then establish a γ-crystallin gradient across the lens to maintain the refractive index[10]. Later studies (Klok et al., 1998) confirmed that indeed, de-methylation increased mRNA and protein levels of γ-crystallin[25]. More recent studies in zebrafish (Tittle et al., 2011) and mouse models (Hoang et al, 2017) confirmed that DNMT1 is necessary for lens development, as inactivation of DNMT1 and subsequent global DNA hypomethylation during embryonic development resulted in apoptosis, reduced proliferation of lens cells, and defects in lens development[11,26]. However, more studies are needed to elucidate the disparate effects (e.g. cellular differentiation, apoptosis, inhibited proliferation) of DNA de-methylation in lens epithelial cells, which may be explained by factors such as the extent of methylation, specific genes targeted, and the stage of development.

More recently, multiple studies further implicated altered DNA methylation in the pathogenesis of age-related cataracts (ARC). Li et al. (2014) compared the DNA methylation profiles of DNA repair genes between age-related cataract tissue and normal lens epithelium and found promoter hypermethylation and reduced mRNA levels of O-6-methylguanine-DNA methyltransferase (MGMT), a DNA repair gene, in the cataract tissue compared to normal lens epithelial tissue[27]. The authors postulated that MGMT methylation and decreased expression contributed to development of cataract. This research group later (in 2015) found that cataract samples also had hypermethylation of 8-oxoguanine DNA glycosylase 1 (OGG1), a base excision repair protein. The researchers were able to demethylate OGG1, which in turn upregulated expression of OGG1 and protected cultured human lens cells against apoptosis induced by UVB light[28]. A 2017 study by the same group identified five other genes (DNMT3B, HDAC, HDAC4, HDAC9, and MBD3) with

increased expression in ARC cells which were postulated to facilitate epigenetic changes in cataracts[29].

3.4. DNA methylation, fibrosis of the trabecular meshwork and glaucoma pathogenesis.

One mechanism that can lead to glaucomatous damage is fibrosis of the trabecular meshwork (TM), which reduces aqueous humor outflow, thereby leading to elevated intraocular pressure (IOP)[30]. This process is mediated by pro-fibrotic factors (TGF-β, platelet-derived growth factor, connective tissue growth factor) that stimulate trabecular cells to produce extracellular structural proteins (e.g. collagen)[31]. Recent studies suggest that epigenetic changes modulate the balance of profibrotic growth factors as well as anti-fibrotic factors, such as RAS protein activator like 1(RASAL1)[30]. In a study using cultured human TM cells, McDonnell et al. (2016) evaluated the DNA methylation profiles of TGF-β1 and RASAL1 in cells from healthy donors and donors with glaucoma. They found distinct methylation profiles between glaucomatous and healthy cells, with glaucomatous TM cells in a pro-fibrotic state, characterized by decreased RASAL1, and increased TGF-β1 and an overall increased DNMT1 expression. Similarly, hypoxia (relative to the physiologic state of low oxygen tension in the anterior chamber) triggered normal TM cells to display similar expression and methylation profiles as the glaucomatous TM cells[32]. However, this *in vitro* model of hypoxia may not be extrapolatable to the *in vivo* microenvironment, characterized by high levels of protective antioxidants and low oxygen tension in the anterior chamber and increased susceptibility to glaucoma when oxygen tension rises[33–35].

Other studies have implicated epigenetic modulation as a potential mechanism for glaucoma pathogenesis. For example, in a study of trabeculectomy sections from glaucoma patients and controls, Chansangpetch et al. (2018) found that the glaucoma group had different methylation profiles of two repetitive genetic sequences – Alu and HERV-K[36]. The hypomethylation of Alu occurred in all subsets of glaucoma (primary open angle, primary closed angle, secondary) so was likely related to the diseased TM itself; meanwhile the hypermethylation of HERV-K was only seen in primary open angle glaucoma, suggesting that it has a more genetic basis. Matsuda et al. (2015) found that dexamethasone treatment of cultured human TM cells, a model for steroid-induced glaucoma, led to demethylation of promoter regions for three genes (FKBP5, ZBTB16, SCNN1A) and methylation of promoter regions for 4 genes (ARSI, HIC1, GREM2, and MATN2), with methylation inversely correlated to gene expression[37]. This steroid-induced methylation causes changes in genes expression and can potentially help elucidate the mechanism behind steroid-induced glaucoma.

4. Post-translational histone modification and anterior segment diseases

Another distinct epigenetic mechanism that regulates gene transcription is through histone modification. Many enzymes, including histone acetyltransferases (HAT), deacetylase (HDACs), and methyltransferases, covalently modify histones via methylation, acetylation, deacetylation, ubiquitination, and phosphorylation[1]. Acetylation and deacetylation alter the balance of electrical charges on the histone, thereby affecting its affinity for binding DNA and chromatin plasticity. In general, acetylation induces relaxation of the chromatin which

increases transcriptional activity, while deacetylation condenses the chromatin and decreases transcription[1,38].

Studies over the past decade have found associations between histone modification and ophthalmic diseases including fibrotic lens complications[39], keratitis[40], age related cataracts[41,42], granular corneal dystrophy[43], and aberrant corneal wound healing[12]. Recent studies of these histone acetylation pathways have led to new therapeutic targets, and numerous histone deacetylase inhibitors (HDACis) are being developed to target these pathways and potentially revolutionize medical treatment[1,39,40,42,44–47].

4.1. Histone deacetylase inhibitors and ocular surface fibrosis and wound healing.

Histone deacetylase inhibitors (HDACis) are a novel group of therapeutics that affect gene expression by increasing acetylation of corresponding histones. Histone deacetylase inhibitors have demonstrated antifibrotic activity in mouse and rat models of hepatic and pulmonary fibrosis, as well as in vitro models of skin fibrosis, by the downregulation of various extracellular matrix-associated genes and inhibition of pro-inflammatory cytokine production[48–52]. Multiple HDACis are undergoing clinical development, including vorinostat, givinostat, abexinostat, belinostat, panobinostat and trichostatin A[53]. Suberoylanilide hydroxamic acid (SAHA) or vorinostat is a HDACi approved by the U.S. Food and Drug Administration (FDA) for use in patients with cutaneous T-cell lymphoma[54]. Whereas other antifibrotic drugs can disrupt normal cellular functions, this HDACi is typically innocuous to corneal fibroblasts and other cells, based on multiple studies[44,54–57]. Recently, Sharma et al. used trichostatin A, in rabbit models of excimer photorefractive keratectomy induced corneal haze and found that this drug was noncytotoxic and decreased corneal haze by inhibiting TGF-beta1 induced myofibroblast formatin[44].

In addition, Sharma et al. (2016) used suberoylanilide hydroxamic acid (SAHA) to inhibit fibrosis in a rabbit model of glaucoma filtering surgery. This study found that following glaucoma filtering surgery, drastically fibrotic postoperative conjunctival healing in rabbits was linked to deacetylation of histones H3 and H4. In turn, SAHA reduced post-operative scarring. In addition, compared to the control group, the eyes treated with SAHA demonstrated increased acetylation of histone H3 and H4, and absence of corneal opacity, neovascularization, and edema[45]. These animal studies demonstrate the therapeutic potential of HDACis as anti-fibrotic agents in ophthalmic conditions, although human studies are needed.

4.2. Histone modification and granular corneal dystrophy and other corneal diseases.

Type 2 granular corneal dystrophy (also known as Avellino corneal dystrophy) is characterized by a mutation in the transforming growth factor β-induced gene, which encodes the extracellular matrix protein TGFβIp. This mutation gradually leads to amyloid and hyaline accumulation in the corneal stroma. TGFβ1 was shown by Maeng et al. (2015) to utilize epigenetic mechanisms to induce the genes encoding amyloid and hyaline. In a study of cultured human corneal fibroblasts, TGFβ1 led to increased histone methylation at the gene promoters (increased monomethylation of the 4th lysine of histone *3*) which also enables transcription factor binding and subsequent upregulated expression of ECM-

producing genes[43]. Thus, histone H3K4me (monomethylation of the 4th lysine of histone *3*) could be a therapeutic target for attenuating expression of ECM and TGFBIp genes in granular corneal dystrophy.

In addition, in a rat model of type I diabetes (utilizing alloxan induction), Herencia-Bueno et al. (2018) found diabetes reduced histone H3 acetylation in the cornea, leading to aberrant chromatin organization[58]. Further research is needed to elucidate the implication of this change in corneas of patients with diabetes.

4.3. Histone modification and age-related cataracts.

It has been established that histone-modifying enzymes are crucial in normal lens development[12]. For instance, early animal models demonstrate the crucial role histone modification plays in ocular disease, including the up-regulation of deacetylases (e.g. SIRT1) in protecting against cataract[59]. Histone modifiers such as the acetyltransferases CBP and p300 have been shown to play a key role in normal cell differentiation in the mammalian lens. In a mouse model, Wolf et al. (2013) inactivated both CBP and p300 in embryonic ectodermal lens cells, causing aphakia and arrested growth into mature lens cells[12].

Histone modification may be triggered by ultraviolet-B (UVB) light exposure, which represses expression of nucleotide excision repair proteins. Wang et al. (2016) found that UVB exposure caused H3K9 deacetylation (and subsequent repression) of ERCC6, which codes the Cockayne syndrome complementation group B (CSB) protein involved in nucleotide excision repair. Without this functioning repair mechanism, cataracts begin to form unchecked. An epigenetic mechanism of age-related nuclear cataract (ARNC) formation is favored over a genetic mechanism of repressed nucleotide excision repair protein expression, as ARNC samples did not share polymorphisms in the ERCC6 gene[41].

In addition, Glutathione S-Transferase Mu 3 (GSTM3) is an antioxidant enzyme that is protective against ARNC formation. Li et al. (2016) found that histone modifications are involved in regulating expression of GSTM3. ARNC patients were found to have transcriptional repression of GSTM3 mediated by hypermethylation of the GSTM3 promoter, as well as deacetylated histone H3 and methylation at histone H3 Lysine 9 (H3K9) – all of which are markers of transcriptional repression. This repression was reversed after treatment with a histone deacetylase inhibitor[42].

Lens fibrosis is characterized by epithelial-mesenchymal transition (EMT) of lens epithelial cells induced by transforming growth factor-β (TGF-β). In a study of fetal human lens epithelial cells, Ganatra et al. (2015) found that TGF-β treatment resulted in increased expression of α-smooth muscle actin (α-SMA), a protein that is also upregulated during EMT. This expression of α-SMA correlated with acetylation of its own histone promoter, thus suggesting that TGF-β leads to histone acetylation of α-SMA which is correlated with lens capsule fibrosis. Trichostatin-A, an inhibitor of HDAC, had the opposite effect and suppressed EMT by decreasing histone H4 acetylation at the α-SMA promoter[39], thereby reducing fibrosis. These findings point toward the exciting future therapeutic options for patients.

5. Chromatin remodeling and anterior segment diseases

In addition to histone modifications, chromatin structure is mediated on a larger scale by remodeling the nucleosomes that contain these histones. In contrast to covalent histone modification, chromatin nucleosome remodeling enzymes utilize ATP hydrolysis to non-covalently move, restructure, and even remove nucleosomes to enable more interaction between the wrapped DNA and transcription machinery[1]. The most thoroughly studied families of chromatin remodelers are SWI/SNF (*switch*/sucrose-non-fermenting), ISWI (imitation *switch)*, INO80 (inositol requiring 80), and CHD (chromodomain-helicase-DNA binding)[60]. Genetic mutations of these remodeling complexes are involved in the pathogenesis of several ocular diseases[40], as seen below. In animal models[61–63], ISWI and SWI/SNF have been found to be crucial for eye development, although human studies are relatively sparse.

5.1. Chromatin remodeling and ocular surface photocarcinogenesis.

Chromatin-remodeling ATPases have been found to regulate angiogenic and inflammatory responses in corneal wound healing. Brahma (BRM), one of the two ATPase subunits of the SWI/SNF chromatin remodeling complex, is a tumor suppressor that is protective against photocarcinogenesis. In a study using mice subjected to radiation, Hassan et al. (2014) found that BRM knockout mice had significantly more hyperplasia and disorganized proliferation of corneal epithelial and stromal cells, while the presence of BRM was protective in control mice[64].

In addition, recent studies (Tsui et al., 2016) have shown that CCCTC binding factor (CTCF)-mediated chromatin remodeling may regulate corneal epithelial cell differentiation by acting as a mediator between transcription factor PAX6 and cell differentiation genes[65].

5.2. Chromatin remodeling and cataracts

In one of the earliest studies on the role of chromatin-remodeling complexes in ocular development, Dirscherl et al. (2005) showed that frog embryos injected with dominant-negative ISWI mutant mRNA (the resulting mutated ATPase results in catalytic inactivation) developed defects in lens development and cataracts[61]. Later studies have concluded that the catalytic subunit BRG1, an APTase of the SWI/SNF chromatin-remodeling complex, is also required for the differentiation of the lens in mice[63] and zebrafish[26]. A mouse model by He et al. (2010) showed that transgenic mice with dysfunctional BRG1 (created by mutating the ATP binding site) developed cataracts with abnormal lens cellular structure[63]. A follow-up study in 2016 identified another chromatin remodeling enzyme, Snf2h, that is required for lens development. Knockdown of Snf2h in transgenic mice caused lens cells to differentiate abnormally, form cataracts, and grow in a stunted and disorganized fashion[13].

5.3. Chromatin remodeling, iris coloration and albinism

In addition to modulating embryonic differentiation, chromatin remodeling complexes are believed to play a role in determining iris color. The remodeling complex helicase-like transcription factor (HLTF), a member of the SWI/SNF family, targets an allele on the oculocutaneous albinism II (OCA2) gene, which encodes a key protein in melanocytes[66]. In

turn, the OCA2 protein in melanocytes leads to brown or blue irides depending on degree of melanin production[67].

6. Non-coding RNA and anterior segment diseases

Non-coding RNAs, otherwise known as RNA interference (RNAi) molecules, regulate expression of genes by attaching to effector enzymes to form complexes that bind to and degrade complementary mRNA[1,68]. Although many types of RNAi molecules exist, microRNAs (miRNAs) have been the most extensively studied, with at least 2,000 human miRNAs identified thus far, many of which regulate basic cellular functions such as inflammation and differentiation[1,69]. In fact, miRNAs are postulated to regulate over 30% of the human genome, primarily by suppressing gene expression[70]. MiRNAs are 22-nucleotide RNA molecules that are first transcribed by RNA polymerase II into primary miRNA. This is followed by processing of the miRNA by the ribonuclease III Drosha into mature miRNA, which then combines with RNA-induced silencing complexes (RISCs) to suppress gene transcription or degrade mRNA[14,71,72].

Small interfering RNAs (siRNAs) are another type of RNAi that similarly combines with RISCs to suppress gene transcription or degrade mRNAs[68,71]. As with miRNA, siRNA is formed by the processing and cleavage of double stranded RNA by RNase III (known as Dicer)[73]. MiRNAs and siRNAs differ mechanistically in that miRNAs are able to bind mRNAs using only some of its nucleotides, whereas siRNAs only bind mRNAs that are complementary to all of its nucleotides. As a result, miRNAs can bind multiple mRNA sequences, whereas siRNAs are highly specific for one mRNA sequence[68].

Although RNAi was first discovered in 1993[74], the first ophthalmic RNA profile wasn't characterized until 2006 using miRNA arrays in adult mice[15]. Since then, although the functions of most miRNAs are still being investigated, normal miRNA expression has been demonstrated as crucial for normal processes including corneal epithelial regeneration[75], wound healing[72], and angiogenesis[76]. Aberrant miRNA expression has been associated with diseases including Sjögren syndrome, ocular surface neoplasias, glaucoma, corneal dystrophy, keratoconus and cataracts[14]. The main interest of researchers has been the therapeutic implications, as siRNAs can theoretically silence overexpressed genes in diseases ranging from cancer to inflammation[71].

6.1. miRNAs and anterior segment diseases

6.1.1 miRNAs and Corneal epithelial healing and corneal angiogenesis—

Since 2006, multiple miRNA profiling studies have demonstrated the tissue-specific expression of miRNA expression, with several of these miRNAs found exclusively in the eye. This ocular miRNA is disproportionately expressed, with 11 of the 378 known miRNAs comprising about 80% of the miRNA expressed in human cornea, ciliary body, and trabecular meshwork samples[14]. The most heavily expressed miRNAs include (mir)-184 expressed in both cornea and lens, mir-204 uniformly expressed in lens epithelial cells, and mir-205 expressed in the cornea[15,77]. Over the past decade, dramatic leaps have also been made in determining miRNAs' role in corneal cell proliferation[78], differentiation[79] and angiogenesis[76].

In a study of human corneal epithelial cells, Lee et al. (2011) found that miR-145 likely regulates immune response and promotes differentiation while suppressing proliferation. In particular, miR-145 suppresses expression of integrin β8 (ITGβ8) while upregulating IFNβ1, which encodes the anti-inflammatory protein interferon-beta. Transfection of corneal epithelial progenitor cells with miR-145 plasmid resulted in a thinner epithelium of atypical morphology (more squamous and fewer cuboidal cells in the basal layer) and fewer cells, as proliferation was suppressed in favor of differentiation[77]. Subsequent studies by the same group (Teng et al., 2015) identified other miRNAs including miR-10b, 126, and 155 that have a variety of functions. They discovered that miRNAs collectively regulate progenitor cell homeostasis by targeting genes encoding transcription factors involved in proliferation and apoptosis, structural proteins such as connexins, and mediators in T cell and B cell receptor signaling[79].

Other MiRNAs such as miR-31, 103, 107, and miR-450b act on distinct pathways to mediate proliferation and differentiation. Peng et al. (2012) found that miR-31, preferentially expressed in the corneal epithelium, promotes differentiation by indirectly upregulating Notch activity, which preferentially leads to differentiation rather than proliferation. MiR-31 inhibits factor-inhibiting hypoxia-inducible factor 1, which normally hydroxylates (and downregulates) the Notch intracellular domain[78]. A follow-up study in 2015 by the same group found that miRs-103 and 107 enhances proliferation through different targets, including kinase p90RSK2, Wnt3a, NEDD9 (HEF1), and tyrosine phosphatase PTPRM[75]. On the other hand, miR-450b stimulates differentiation of limbal cells by repressing the SOX2/P63 pathway[80].

In addition to regulating cellular differentiation and proliferation, miRNAs also regulate wound healing and angiogenesis. A recent study by Park et al. (2017) showed that miR-184 downregulates corneal angiogenesis by targeting transcriptional regulators that downregulate the Akt/VEGF pathway, thereby suppressing the metalloproteinases needed for angiogenesis. Expression of miR-184 in human epithelial cells reduced the healing ability and neovascularization in nearby dermal cells[76]. An earlier study by An et al. (2015) showed that corneal wound healing in mice involved changes in expression of 29 miRNAs, especially miR-204, which was increased during wound healing by 267-fold. Transfection of miR-204 into human corneal epithelial cells dramatically reduced cellular proliferation[72].

6.1.2. miRNAs and other corneal diseases—MiRNAs are vital for corneal metabolism, differentiation, and proliferation. As such, embryonic aberrancies in miRNA expression can lead to corneal dysgenesis, while later disruptions in miRNA expression may cause corneal opacities and blindness. A variety of microRNAs have been implicated in corneal diseases. For example, mutations in miR-184 may be linked to keratoconus with cataracts due to inadequate binding to 3’ untranslated regions (UTRs), which can regulate translation in regulatory genes[14]. Numerous miRNAs are involved in the pathogenesis of HSV keratitis, including miR-132 and miR-155. In a mouse model, ocular HSV infection significantly upregulated miR-132, with an associated rise in VEGF and IL-17. Knockdown of miR-132 reduced corneal angiogenesis and inflammation in ocular HSV[81]. A later study found that silencing MiR-155 produced similar results, with these mice exhibiting attenuations in T helper cell response, angiogenesis, and lesion severity[82]. In addition,

miR-10b, miR-146, and dozens of other miRNAs have been identified in the pathogenesis of ocular manifestations in diabetes. Diabetes upregulates miR-10b and miR-146a, which were found to increase corneal epithelial proliferation[83] and impair wound healing and migration, respectively[84].

6.1.3. miRNAs and glaucoma—MiRNA expression plays an essential role in the pathogenesis of ocular diseases such as glaucoma[14]. Glaucoma has been linked to impaired TM contractibility and collagen deposition. Multiple miRNAs (MiR-200c, MiR-155, MiR-204) inhibit TM contractibility, while miR-29 downregulates collagen-producing genes and TGFβ1. Increased oxygenation was shown to induce oxidative stress and downregulate miR-29b while upregulating collagen-producing genes. This was partially reversed by transfection with miR-29b mimics. Furthermore, iatrogenic mechanical stress upregulated miRNAs (e.g. miR-106b, miR-16, miR-26a, miR-27a, miR-27b, and miR-7) thought to play a role in fibrosis in TM cells[85].

6.1.4. miRNAs and cataract—Research thus far on miRNA involvement in lens pathologies focuses on the epithelial-to-mesenchymal transition (EMT), lens epithelial cell proliferation, and fibrosis. In the development of secondary cataracts or posterior capsular opacifications, lens fibrosis involves TGFβ2-stimulated EMT, which is stimulated by activation of Jagged-1/Notch signaling. LECs transfected with miR-26a and miR-26b inhibitors showed increased proliferation, while overexpression of miR-26a and 26b inhibited proliferation and downregulated collagen-synthesizing genes such as alpha-SMA and Col I. By transfecting miR-26a and 26b into cells that had mutant or normal Jagged-1 binding sites, miR-26a and -26b were found to directly target Jagged-1 and suppress Jagged-1/Notch signaling as evidenced by a decrease in Jagged-1 mRNA and protein. In turn, EMT and lens fibrosis could be reversed in lens epithelial cells[86].

6.2. siRNAs and anterior segment diseases

6.2.1 siRNAs and dry eye disease—A novel siRNA, Tivanisiran, has shown promise for symptomatic relief of dry eye disease and is currently undergoing phase I and II clinical trials. This siRNA acts by binding and degrading complementary Transient Receptor Potential Vanilloid 1 (TRPV1) mRNA, which acts as pain receptor in corneal nerve fibers. TRPV1 is expressed in primary afferent nociceptive neuros and is abundant in various eye tissues and it is activated by hyperosmolarity (as seen in dry eye disease), corneal damage and inflammatory mediators. Studies have shown that Tivanisiran has a high specificity for TRPV1 and decreases TRPV1 mRNA by up to 60%[87].

6.2.2. siRNAs and post-surgical conjunctival fibrosis—Conjunctival fibrosis is partly mediated by the myocardin-related transcription factor/Serum response factor (MRTF/SRF) pathway, which leads to fibroblast activation. Thus, research has focused on siRNAs to silence the MRTF gene to prevent fibrosis after glaucoma filtration surgery. Although effective siRNAs have been developed, siRNA delivery to the gene still presents a challenge. Recent trials have shown promise – in vitro, receptor-targeted liposome-peptide-siRNA nanoparticles can effectively deliver siRNA and silence the MRTF-B gene by up to 76%[88].

6.2.3. siRNAs and posterior capsular opacification—Posterior capsular opacification (PCO) is a frequent complication of extracapsular cataract surgery, resulting from excessive lens epithelial cell (LEC) proliferation and migration which is mediated by EMT and upregulated cytokines. Accordingly, the suppression of LEC proliferation and EMT have been studied as therapeutic interventions. At least two pathways that induce EMT have been identified as targets of RNAi molecules[89,90]. The first pathway involves transforming growth factor-β2 (TGF-β2), Smad2, and Smad3, which have been found to be inhibited by miR-486–5p. Human LECs that overexpressed MiR-486–5p had reduced wound healing, proliferation, EMT, and migration compared to controls[90]. The second pathway involves targeting and inhibiting mammalian target of rapamycin (mTOR), p70 ribosomal protein S6 kinase (p70S6K), and protein kinase B (PKB or Akt), which are all elevated in LEC proliferation. In a human LEC B3 cell model, transfection of siRNA of mTOR (simTOR) inhibited mTOR/p70S6K/Akt and suppressed expression of mTOR complex 1 (mTORC1), mTORC2, and TGF-β-induced EMT[89].

7. Epigenetic therapies

Epigenetic therapy targets specific regulatory mechanisms, which minimizes the risk of complications that can be seen with systemic agents. Therapeutic targets have already been identified, including altered DNA methylation in Behcet's disease[91] and retinoblastoma[92], histone modification in uveitis[59] and lens and conjunctival fibrosis[39,88], and siRNA expression in dry eye disease[87]. Though this next generation of therapeutics holds significant promise for the future of medicine, challenges such as bioavailability and targeted drug delivery are still being addressed.

Several epigenetic drugs are already on the market, including histone deacetylase inhibitors (HDACi) such as vorinostat and RNAi therapeutics such as patisiran. Although these epigenetic treatments have been shown to be effective in systemic diseases such as cancer and polyneuropathy, epigenetic pharmaceuticals for ophthalmic diseases are still undergoing clinical trials. Histone deacetylase inhibitors have already shown promise in preventing corneal scarring[45]. Although the only FDA-approved HDACIs on the market are for treating cancer, HDACIs for ophthalmic indications are undergoing clinical trials[38,45].

A main challenge to applying epigenetic, and particularly RNAi-based, therapies is adequate delivery to target cells, as RNAi molecules can decay intravenously, exit out the kidneys, or fail to enter the plasma membrane[73,93]. Over the past decade, researchers developed more effective formulations for delivery, such as encapsulation in lipid nanoparticles[93]. Advances like these have eased more than a dozen RNAi therapeutic programs into clinical trials targeting diseases ranging from fibrosis to cancer. For ophthalmic disease, the challenge of delivery can be conveniently circumvented by localized injection of siRNA[73]. At least eight of the RNAi therapeutic programs target ophthalmic disease[68]. The first FDA-approved RNAi therapeutic, patisiran, became available in 2018 for treatment of hereditary transthyretin-mediated amyloidosis, and RNAi therapies are currently undergoing testing to treat a host of ophthalmic diseases including age-related macular degeneration, proliferative vitreoretinopathy, ocular hypertension, and dry eye diseases[68,71,87]. Admittedly, fewer studies have explored RNAi therapies for diseases of the anterior eye segment[68].

RNAi therapies treating anterior ophthalmic disease avoids the challenges of targeted delivery and bioavailability present for other indications because of the easy accessibility and sequestered structure of the anterior ocular segment[94]. Notably, a recently developed reducible branched polyethylenimine (rBPEI)-based nanoparticle (NP) system was able to deliver siRNA throughout the cornea to treat corneal neovascularization in rats[73,95]. Although challenges such as cytotoxicity must be addressed before anterior segment siRNAs can become readily available, their potential has not been lost on pharmaceutical companies and researchers, who are investigating RNAi therapies for herpetic keratitis and corneal and conjunctival scarring[71,88]. It is evident that a number of previously difficult challenges are on the verge of being overcome, making epigenetic therapeutics, particularly in the anterior segment, an exciting and rapidly changing field of research.

8. Conclusion and future directions

Recent technological advances and discoveries have sparked a surge of interest in the integral role of epigenetics in ocular disease. Advances in sequencing and bioinformatics have facilitated discoveries on the role of epigenetics in ocular disease pathogenesis and facilitated a realization of their potential in next generation treatment therapies.

Epigenetic therapy has distinct advantages over traditional medications, such as increased specificity and a reduced side effect profile. Although some epigenetic medications have been shown to be effective in systemic diseases such as cancer, epigenetic therapy for ophthalmic disease are still undergoing clinical trials. There are still broad gaps in our knowledge regarding the mechanistic link between epigenetic changes and ocular disease, which present challenges to therapeutically targeting meaningful epigenetic changes that are causes rather than side effects of the disease itself. However, epigenetic therapies including RNAi molecules and Bromodomain and Extra-Terminal motif (BET) inhibitors (e.g. JQ1) have shown potential in treating autoimmune keratitis, corneal wound healing, and ocular inflammation respectively[46].

Innovation over the past decade has identified many new epigenetic modifiers as potential therapeutic targets, such as altered DNA methylation in Fuchs' dystrophy[16]. Future research in epigenetics can further our knowledge of ocular disease pathogenesis and revolutionize the way we treat ocular disease. More studies are needed to identify therapeutic targets and biomarkers, elucidate the precise mechanisms responsible for epigenetics on disease pathogenesis, assess the safety and efficacy of novel epigenetic therapies, and introduce these treatments to the market.

Financial support and conflicts of interest

This work was supported by the grant NIH K12EY021475. There is no conflict of interest to declare.

References

1. Voyias PD, Patel A, Arasaradnam RP. Epigenetic Biomarkers of Disease In: Medical Epigenetics. Elsevier; 2016:159–176. doi:10.1016/B978-0-12-803239-8.00010-7
2. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001;414(6859):105–111. doi:10.1038/35102167 [PubMed: 11689955]

3. Cheung TH, Rando TA. Molecular regulation of stem cell quiescence. Nat Rev Mol Cell Biol. 2013;14(6):329–340. doi:10.1038/nrm3591 [PubMed: 23698583]
4. Casadevall D, Kilian AY, Bellmunt J. The prognostic role of epigenetic dysregulation in bladder cancer: A systematic review. Cancer Treat Rev. 2017;61:82–93. doi:10.1016/j.ctrv.2017.10.004 [PubMed: 29121502]
5. Wang T, Chuffart F, Bourova-Flin E, et al. Histone variants: critical determinants in tumour heterogeneity. Front Med. 10 2018. doi:10.1007/s11684-018-0667-3
6. Zhang W, Song M, Qu J, Liu G-H. Epigenetic Modifications in Cardiovascular Aging and Diseases. Circ Res. 2018;123(7):773–786. doi:10.1161/CIRCRESAHA.118.312497 [PubMed: 30355081]
7. Kwon Min Jee, Kim Sunhong, Han Myeong Hoon, Lee Sung Bae. Epigenetic Changes in Neurodegenerative Diseases. Mol Cells. 2016;39(11):783–789. doi:10.14348/MOLCELLS.2016.0233 [PubMed: 27871175]
8. Wang Y, Guan H. The Role of DNA Methylation in Lens Development and Cataract Formation. Cell Mol Neurobiol. 2017;37(6):979–984. doi:10.1007/s10571-016-0447-z [PubMed: 27858287]
9. Bonnin N, Belville C, Chiambaretta F, Sapin V, Blanchon L. DNA methyl transferases are differentially expressed in the human anterior eye segment. Acta Ophthalmol (Copenh). 2014;92(5):e366–e371. doi:10.1111/aos.12365
10. Peek R, Niessen RWLM, Schoenmakers JGG, Lubsen NH. DNA methylation as a regulatory mechanism in rat γ-crystallin gene expression. Nucleic Acids Res. 1991;19(1):77–83. doi:10.1093/nar/19.1.77 [PubMed: 2011513]
11. Hoang TV, Horowitz ER, Chaffee BR, et al. Lens development requires DNMT1 but takes place normally in the absence of both DNMT3A and DNMT3B activity. Epigenetics. 2017;12(1):27–40. doi:10.1080/15592294.2016.1253651 [PubMed: 27824296]
12. Wolf L, Harrison W, Huang J, et al. Histone posttranslational modifications and cell fate determination: lens induction requires the lysine acetyltransferases CBP and p300. Nucleic Acids Res. 2013;41(22):10199–10214. doi:10.1093/nar/gkt824 [PubMed: 24038357]
13. He S, Limi S, McGreal RS, et al. Chromatin remodeling enzyme Snf2h regulates embryonic lens differentiation and denucleation. Development. 2016;143(11):1937–1947. doi:10.1242/dev.135285 [PubMed: 27246713]
14. Drewry M, Helwa I, Allingham RR, Hauser MA, Liu Y. miRNA Profile in Three Different Normal Human Ocular Tissues by miRNA-Seq. Investig Opthalmology Vis Sci. 2016;57(8):3731. doi:10.1167/iovs.16-19155
15. Ryan DG, Oliveira-Fernandes M, Lavker RM. MicroRNAs of the mammalian eye display distinct and overlapping tissue specificity. Mol Vis. 2006;12:1175–1184. [PubMed: 17102797]
16. Khuc E, Bainer R, Wolf M, et al. Comprehensive characterization of DNA methylation changes in Fuchs endothelial corneal dystrophy. Yu F-S, ed. PLOS ONE. 2017;12(4):e0175112. doi:10.1371/journal.pone.0175112 [PubMed: 28384203]
17. Cho H, Sobrin L. Genetics of Diabetic Retinopathy. Curr Diab Rep. 2014;14(8):515. doi:10.1007/s11892-014-0515-z [PubMed: 24952107]
18. Hammond CJ, Snieder H, Gilbert CE, Spector TD. Genes and environment in refractive error: the twin eye study. Invest Ophthalmol Vis Sci. 2001;42(6):1232–1236. [PubMed: 11328732]
19. Ling C, Groop L. Epigenetics: A Molecular Link Between Environmental Factors and Type 2 Diabetes. Diabetes. 2009;58(12):2718–2725. doi:10.2337/db09-1003 [PubMed: 19940235]
20. Grazioli E, Dimauro I, Mercatelli N, et al. Physical activity in the prevention of human diseases: role of epigenetic modifications. BMC Genomics. 2017;18(S8):802. doi:10.1186/s12864-017-4193-5 [PubMed: 29143608]
21. Li E, Zhang Y. DNA Methylation in Mammals. Cold Spring Harb Perspect Biol. 2014;6(5):a019133–a019133. doi:10.1101/cshperspect.a019133 [PubMed: 24789823]
22. Luo G, Jing X, Yang S, et al. DNA Methylation Regulates Corneal Epithelial Wound Healing by Targeting miR-200a and CDKN2B. Investig Opthalmology Vis Sci. 2019;60(2):650. doi:10.1167/iovs.18-25443
23. Hamill CE, Schmedt T, Jurkunas U. Fuchs Endothelial Cornea Dystrophy: A Review of the Genetics Behind Disease Development. Semin Ophthalmol. 2013;28(5–6):281–286. doi:10.3109/08820538.2013.825283 [PubMed: 24138036]

24. Afshari NA, Li Y-J, Pericak-Vance MA, Gregory S, Klintworth GK. Genome-wide Linkage Scan in Fuchs Endothelial Corneal Dystrophy. Investig Opthalmology Vis Sci. 2009;50(3):1093. doi:10.1167/iovs.08-1839
25. Klok EJ, van Genesen ST, Civil A, Schoenmakers JGG, Lubsen NH. Regulation of Expression within a Gene Family: THE CASE OF THE RAT γB- AND γD-CRYSTALLIN PROMOTERS. J Biol Chem. 1998;273(27):17206–17215. doi:10.1074/jbc.273.27.17206 [PubMed: 9642290]
26. Tittle RK, Sze R, Ng A, et al. Uhrf1 and Dnmt1 are required for development and maintenance of the zebrafish lens. Dev Biol. 2011;350(1):50–63. doi:10.1016/j.ydbio.2010.11.009 [PubMed: 21126517]
27. Li F, Wang Y, Zhang G, Zhou J, Yang L, Guan H. Expression and methylation of DNA repair genes in lens epithelium cells of age-related cataract. Mutat Res Mol Mech Mutagen. 2014;766–767:31–36. doi:10.1016/j.mrfmmm.2014.05.010
28. Wang Y, Li F, Zhang G, Kang L, Qin B, Guan H. Altered DNA Methylation and Expression Profiles of 8-Oxoguanine DNA Glycosylase 1 in Lens Tissue from Age-related Cataract Patients. Curr Eye Res. 2015;40(8):815–821. doi:10.3109/02713683.2014.957778 [PubMed: 25310012]
29. Wang Y, Zhang G, Kang L, Guan H. Expression Profiling of DNA Methylation and Transcriptional Repression Associated Genes in Lens Epithelium Cells of Age-Related Cataract. Cell Mol Neurobiol. 2017;37(3):537–543. doi:10.1007/s10571-016-0393-9 [PubMed: 27306760]
30. McDonnell FS, McNally SA, Clark AF, O'Brien CJ, Wallace DM. Increased Global DNA Methylation and Decreased TGFβ1 Promoter Methylation in Glaucomatous Lamina Cribrosa Cells: J Glaucoma. 2016;25(10):e834–e842. doi:10.1097/IJG.0000000000000453 [PubMed: 27300643]
31. Zhavoronkov A, Izumchenko E, Kanherkar RR, et al. Pro-fibrotic pathway activation in trabecular meshwork and lamina cribrosa is the main driving force of glaucoma. Cell Cycle. 2016;15(12):1643–1652. doi:10.1080/15384101.2016.1170261 [PubMed: 27229292]
32. McDonnell F, Irnaten M, Clark AF, O'Brien CJ, Wallace DM. Hypoxia-Induced Changes in DNA Methylation Alter RASAL1 and TGFβ1 Expression in Human Trabecular Meshwork Cells. Wong C-M, ed. PLOS ONE. 2016;11(4):e0153354. doi:10.1371/journal.pone.0153354 [PubMed: 27124111]
33. Siegfried CJ, Shui Y-B. Intraocular Oxygen and Antioxidant Status: New Insights on the Effect of Vitrectomy and Glaucoma Pathogenesis. Am J Ophthalmol. 2019;203:12–25. doi:10.1016/j.ajo.2019.02.008 [PubMed: 30772349]
34. Holekamp NM, Shui Y-B, Beebe DC. Vitrectomy surgery increases oxygen exposure to the lens: A possible mechanism for nuclear cataract formation. Am J Ophthalmol. 2005;139(2):302–310. doi:10.1016/j.ajo.2004.09.046 [PubMed: 15733992]
35. Helbig H, Hinz JP, Kellner U, Foerster MH. Oxygen in the anterior chamber of the human eye. Ger J Ophthalmol. 1993;2(3):161–164. [PubMed: 8334391]
36. Chansangpetch S, Prombhul S, Tantisevi V, et al. DNA Methylation Status of the Interspersed Repetitive Sequences for LINE-1, Alu, HERV-E, and HERV-K in Trabeculectomy Specimens from Glaucoma Eyes. J Ophthalmol. 2018;2018:1–10. doi:10.1155/2018/9171536
37. Matsuda A, Asada Y, Takakuwa K, Sugita J, Murakami A, Ebihara N. DNA Methylation Analysis of Human Trabecular Meshwork Cells During Dexamethasone Stimulation. Investig Opthalmology Vis Sci. 2015;56(6):3801. doi:10.1167/iovs.14-16008
38. Liu N, Li S, Wu N, Cho K-S. Acetylation and deacetylation in cancer stem-like cells. Oncotarget. 2017;8(51). doi:10.18632/oncotarget.19167
39. Ganatra DA, Rajkumar S, Patel AR, et al. Association of histone acetylation at the ACTA2 promoter region with epithelial mesenchymal transition of lens epithelial cells. Eye. 2015;29(6):828–838. doi:10.1038/eye.2015.29 [PubMed: 25853442]
40. Yan B, Yao J, Tao Z-F, Jiang Q. Epigenetics and Ocular Diseases: From Basic Biology to Clinical Study: EPIGENETICS IN OPHTHALMOLOGY. J Cell Physiol. 2014;229(7):825–833. doi:10.1002/jcp.24522 [PubMed: 24318407]
41. Wang Y, Li F, Zhang G, Kang L, Guan H. Ultraviolet-B induces ERCC6 repression in lens epithelium cells of age-related nuclear cataract through coordinated DNA hypermethylation and

histone deacetylation. Clin Epigenetics. 2016;8(1):62. doi:10.1186/s13148-016-0229-y [PubMed: 27231489]

42. Li B, Zhou J, Zhang G, et al. Relationship Between the Altered Expression and Epigenetics of GSTM3 and Age-Related Cataract. Investig Opthalmology Vis Sci. 2016;57(11):4721. doi:10.1167/iovs.16-19242
43. Maeng Y-S, Lee G-H, Choi S-I, Kim KS, Kim EK. Histone methylation levels correlate with TGFBIp and extracellular matrix gene expression in normal and granular corneal dystrophy type 2 corneal fibroblasts. BMC Med Genomics. 2015;8(1):74. doi:10.1186/s12920-015-0151-8 [PubMed: 26553048]
44. Sharma A, Mehan MM, Sinha S, Cowden JW, Mohan RR. Trichostatin A Inhibits Corneal Haze In Vitro and In Vivo. Investig Opthalmology Vis Sci. 2009;50(6):2695. doi:10.1167/iovs.08-2919
45. Sharma A, Anumanthan G, Reyes M, et al. Epigenetic Modification Prevents Excessive Wound Healing and Scar Formation After Glaucoma Filtration Surgery. Investig Opthalmology Vis Sci. 2016;57(7):3381. doi:10.1167/iovs.15-18750
46. Ratay ML, Balmert SC, Bassin EJ, Little SR. Controlled release of an HDAC inhibitor for reduction of inflammation in dry eye disease. Acta Biomater. 2018;71:261–270. doi:10.1016/j.actbio.2018.03.002 [PubMed: 29526828]
47. Zhang H, Dai X, Qi Y, He Y, Du W, Pang J. Histone Deacetylases Inhibitors in the Treatment of Retinal Degenerative Diseases: Overview and Perspectives. J Ophthalmol. 2015;2015:1–9. doi:10.1155/2015/250812
48. Korfei M, Stelmaszek D, MacKenzie B, et al. Comparison of the antifibrotic effects of the pan-histone deacetylase-inhibitor panobinostat versus the IPF-drug pirfenidone in fibroblasts from patients with idiopathic pulmonary fibrosis. Zissel G, ed. PLOS ONE. 2018;13(11):e0207915. doi:10.1371/journal.pone.0207915 [PubMed: 30481203]
49. Hemmatazad H, Rodrigues HM, Maurer B, et al. Histone deacetylase 7, a potential target for the antifibrotic treatment of systemic sclerosis. Arthritis Rheum. 2009;60(5):1519–1529. doi:10.1002/art.24494 [PubMed: 19404935]
50. Sanders YY, Hagood JS, Liu H, Zhang W, Ambalavanan N, Thannickal VJ. Histone deacetylase inhibition promotes fibroblast apoptosis and ameliorates pulmonary fibrosis in mice. Eur Respir J. 2014;43(5):1448–1458. doi:10.1183/09031936.00095113 [PubMed: 24603818]
51. Park KC, Park JH, Jeon JY, et al. A new histone deacetylase inhibitor improves liver fibrosis in BDL rats through suppression of hepatic stellate cells: Suppression of hepatic fibrosis. Br J Pharmacol. 2014;171(21):4820–4830. doi:10.1111/bph.12590 [PubMed: 24467283]
52. Wang Y, Zhao L, Jiao F-Z, Zhang W-B, Chen Q, Gong Z-J. Histone deacetylase inhibitor suberoylanilide hydroxamic acid alleviates liver fibrosis by suppressing the transforming growth factor-β1 signal pathway. Hepatobiliary Pancreat Dis Int. 2018;17(5):423–429. doi:10.1016/j.hbpd.2018.09.013 [PubMed: 30249543]
53. Eckschlager T, Plch J, Stiborova M, Hrabeta J. Histone Deacetylase Inhibitors as Anticancer Drugs. Int J Mol Sci. 2017;18(7):1414. doi:10.3390/ijms18071414
54. Duvic M, Vu J. Vorinostat: a new oral histone deacetylase inhibitor approved for cutaneous T-cell lymphoma. Expert Opin Investig Drugs. 2007;16(7):1111–1120. doi:10.1517/13543784.16.7.1111
55. Li X, Zhou Q, Hanus J, et al. Inhibition of Multiple Pathogenic Pathways by Histone Deacetylase Inhibitor SAHA in a Corneal Alkali-Burn Injury Model. Mol Pharm. 2013;10(1):307–318. doi:10.1021/mp300445a [PubMed: 23186311]
56. Gronkiewicz KM, Giuliano EA, Sharma A, Mohan RR. Molecular mechanisms of suberoylanilide hydroxamic acid in the inhibition of TGF-β1-mediated canine corneal fibrosis. Vet Ophthalmol. 2016;19(6):480–487. doi:10.1111/vop.12331 [PubMed: 26559782]
57. Tandon A, Tovey JCK, Waggoner MR, et al. Vorinostat: A Potent Agent to Prevent and Treat Laser-induced Corneal Haze. J Refract Surg. 2012;28(4):285–290. doi:10.3928/1081597X-20120210-01 [PubMed: 22386369]
58. Herencia-Bueno KE, Aldrovani M, Crivelaro RM, et al. Reduction in Histone H3 Acetylation and Chromatin Remodeling in Corneas of Alloxan-Induced Diabetic Rats: Cornea. 2018;37(5):624–632. doi:10.1097/ICO.0000000000001533 [PubMed: 29384804]

59. Gardner PJ, Joshi L, Lee RWJ, Dick AD, Adamson P, Calder VL. SIRT1 activation protects against autoimmune T cell-driven retinal disease in mice via inhibition of IL-2/Stat5 signaling. J Autoimmun. 2013;42:117–129. doi:10.1016/j.jaut.2013.01.011 [PubMed: 23395551]
60. Becker PB, Workman JL. Nucleosome Remodeling and Epigenetics. Cold Spring Harb Perspect Biol. 2013;5(9):a017905–a017905. doi:10.1101/cshperspect.a017905 [PubMed: 24003213]
61. Dirscherl SS, Henry JJ, Krebs JE. Neural and eye-specific defects associated with loss of the Imitation Switch (ISWI) chromatin remodeler in Xenopus laevis. Mech Dev. 2005;122(11):1157–1170. doi:10.1016/j.mod.2005.08.002 [PubMed: 16169710]
62. Hensley MR, Emran F, Bonilla S, et al. Cellular Expression of Smarca4 (Brg1)-regulated Genes in Zebrafish Retinas. BMC Dev Biol. 2011;11(1):45. doi:10.1186/1471-213X-11-45 [PubMed: 21756345]
63. He S, Pirity MK, Wang W-L, et al. Chromatin remodeling enzyme Brg1 is required for mouse lens fiber cell terminal differentiation and its denucleation. Epigenetics Chromatin. 2010;3(1):21. doi:10.1186/1756-8935-3-21 [PubMed: 21118511]
64. Hassan NMM, Painter N, Howlett CR, et al. Brm Inhibits the Proliferative Response of Keratinocytes and Corneal Epithelial Cells to Ultraviolet Radiation-Induced Damage. Haass NK, ed. PLoS ONE. 2014;9(9):e107931. doi:10.1371/journal.pone.0107931 [PubMed: 25254962]
65. Tsui S, Wang J, Wang L, Dai W, Lu L. CTCF-Mediated and Pax6-Associated Gene Expression in Corneal Epithelial Cell-Specific Differentiation. Mantovani R, ed. PLOS ONE. 2016;11(9):e0162071. doi:10.1371/journal.pone.0162071 [PubMed: 27583466]
66. Zhu G, Evans DM, Duffy DL, et al. A Genome Scan for Eye Color in 502 Twin Families: Most Variation is due to a QTL on Chromosome 15q. Twin Res. 2004;7(2):197–210. doi:10.1375/136905204323016186 [PubMed: 15169604]
67. Sturm RA, Larsson M. Genetics of human iris colour and patterns. Pigment Cell Melanoma Res. 2009;22(5):544–562. doi:10.1111/j.1755-148X.2009.00606.x [PubMed: 19619260]
68. Chakraborty C, Sharma AR, Sharma G, Doss CGP, Lee S-S. Therapeutic miRNA and siRNA: Moving from Bench to Clinic as Next Generation Medicine. Mol Ther - Nucleic Acids. 2017;8:132–143. doi:10.1016/j.omtn.2017.06.005 [PubMed: 28918016]
69. Alles J, Fehlmann T, Fischer U, et al. An estimate of the total number of true human miRNAs. Nucleic Acids Res. 2019;47(7):3353–3364. doi:10.1093/nar/gkz097 [PubMed: 30820533]
70. Lewis BP, Burge CB, Bartel DP. Conserved Seed Pairing, Often Flanked by Adenosines, Indicates that Thousands of Human Genes are MicroRNA Targets. Cell. 2005;120(1):15–20. doi:10.1016/j.cell.2004.12.035 [PubMed: 15652477]
71. Tam C, Wong JH, Cheung RCF, Zuo T, Ng TB. Therapeutic potentials of short interfering RNAs. Appl Microbiol Biotechnol. 2017;101(19):7091–7111. doi:10.1007/s00253-017-8433-z [PubMed: 28791440]
72. An J, Chen X, Chen W, et al. MicroRNA Expression Profile and the Role of miR-204 in Corneal Wound Healing. Investig Opthalmology Vis Sci. 2015;56(6):3673. doi:10.1167/iovs.15-16467
73. Pan M, Ni J, He H, Gao S, Duan X. New paradigms on siRNA local application. BMB Rep. 2015;48(3):147–152. doi:10.5483/BMBRep.2015.48.3.089 [PubMed: 25081998]
74. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993;75(5):843–854. doi:10.1016/0092-8674(93)90529-Y [PubMed: 8252621]
75. Peng H, Park JK, Katsnelson J, et al. microRNA-103/107 Family Regulates Multiple Epithelial Stem Cell Characteristics: microRNAs-103/107 and Epithelial Stem Cells. STEM CELLS. 2015;33(5):1642–1656. doi:10.1002/stem.1962 [PubMed: 25639731]
76. Park JK, Peng H, Yang W, Katsnelson J, Volpert O, Lavker RM. miR-184 exhibits angiostatic properties *via* regulation of Akt and VEGF signaling pathways. FASEB J. 2017;31(1):256–265. doi:10.1096/fj.201600746R [PubMed: 27825105]
77. Lee SK-W, Teng Y, Wong H-K, et al. MicroRNA-145 Regulates Human Corneal Epithelial Differentiation. Rojas M, ed. PLoS ONE. 2011;6(6):e21249. doi:10.1371/journal.pone.0021249 [PubMed: 21701675]

78. Peng H, Kaplan N, Hamanaka RB, et al. microRNA-31/factor-inhibiting hypoxia-inducible factor 1 nexus regulates keratinocyte differentiation. Proc Natl Acad Sci. 2012;109(35):14030–14034. doi:10.1073/pnas.1111292109 [PubMed: 22891326]

79. Teng Y, Wong HK, Jhanji V, et al. Signature microRNAs in human cornea limbal epithelium. Funct Integr Genomics. 2015;15(3):277–294. doi:10.1007/s10142-014-0417-9 [PubMed: 25487418]

80. Bhattacharya S, Serror L, Nir E, et al. SOX2 Regulates P63 and Stem/Progenitor Cell State in the Corneal Epithelium: SOX2 Regulates P63 and Corneal Stem/Progenitor Cells. STEM CELLS. 2019;37(3):417–429. doi:10.1002/stem.2959 [PubMed: 30548157]

81. Mulik S, Xu J, Reddy PBJ, et al. Role of miR-132 in Angiogenesis after Ocular Infection with Herpes Simplex Virus. Am J Pathol. 2012;181(2):525–534. doi:10.1016/j.ajpath.2012.04.014 [PubMed: 22659469]

82. Bhela S, Mulik S, Gimenez F, et al. Role of miR-155 in the Pathogenesis of Herpetic Stromal Keratitis. Am J Pathol. 2015;185(4):1073–1084. doi:10.1016/j.ajpath.2014.12.021 [PubMed: 25700796]

83. Kulkarni M, Leszczynska A, Wei G, et al. Genome-wide analysis suggests a differential microRNA signature associated with normal and diabetic human corneal limbus. Sci Rep. 2017;7(1):3448. doi:10.1038/s41598-017-03449-7 [PubMed: 28615632]

84. Winkler MA, Dib C, Ljubimov AV, Saghizadeh M. Targeting miR-146a to Treat Delayed Wound Healing in Human Diabetic Organ-Cultured Corneas. Yu F-S, ed. PLoS ONE. 2014;9(12):e114692. doi:10.1371/journal.pone.0114692 [PubMed: 25490205]

85. Gonzalez P, Li G, Qiu J, Wu J, Luna C. Role of MicroRNAs in the Trabecular Meshwork. J Ocul Pharmacol Ther. 2014;30(2–3):128–137. doi:10.1089/jop.2013.0191 [PubMed: 24383444]

86. Chen X, Xiao W, Chen W, et al. MicroRNA-26a and -26b inhibit lens fibrosis and cataract by negatively regulating Jagged-1/Notch signaling pathway: This article has been corrected since Online Publication and an erratum has also been Published. Cell Death Differ. 2017;24(8):1431–1442. doi:10.1038/cdd.2016.152 [PubMed: 28622289]

87. Moreno-Montañés J, Bleau A-M, Jimenez AI. Tivanisiran, a novel siRNA for the treatment of dry eye disease. Expert Opin Investig Drugs. 2018;27(4):421–426. doi:10.1080/13543784.2018.1457647

88. Yu-Wai-Man C, Tagalakis AD, Manunta MD, Hart SL, Khaw PT. Receptor-targeted liposome-peptide-siRNA nanoparticles represent an efficient delivery system for MRTF silencing in conjunctival fibrosis. Sci Rep. 2016;6(1):21881. doi:10.1038/srep21881 [PubMed: 26905457]

89. Zhang C, Liu J, Jin N, Zhang G, Xi Y, Liu H. SiRNA Targeting mTOR Effectively Prevents the Proliferation and Migration of Human Lens Epithelial Cells. Nagaraj R, ed. PLOS ONE. 2016;11(12):e0167349. doi:10.1371/journal.pone.0167349 [PubMed: 27911920]

90. Liu B, Sun J, Lei X, Zhu Z, Pei C, Qin L. MicroRNA-486–5p suppresses TGF-β2-induced proliferation, invasion and epithelial–mesenchymal transition of lens epithelial cells by targeting Smad2. J Biosci. 2017;42(4):575–584. doi:10.1007/s12038-017-9709-2 [PubMed: 29229876]

91. Zhu Y, Qiu Y, Yu H, et al. Aberrant DNA methylation of GATA binding protein 3 (GATA3), interleukin-4 (IL-4), and transforming growth factor-β (TGF-β) promoters in Behcet's disease. Oncotarget. 2017;8(38). doi:10.18632/oncotarget.19500

92. Berdasco M, Gómez A, Rubio MJ, et al. DNA Methylomes Reveal Biological Networks Involved in Human Eye Development, Functions and Associated Disorders. Sci Rep. 2017;7(1):11762. doi:10.1038/s41598-017-12084-1 [PubMed: 28924151]

93. Ledford H. G ene-silencing technology gets first drug approval after 20-year wait. Nature. 2018;560(7718):291–292. doi:10.1038/d41586-018-05867-7 [PubMed: 30108348]

94. Turchinovich A, Zoidl G, Dermietzel R. RNAi in Ocular Diseases In: Cheng K, Mahato RI, eds. Advanced Delivery and Therapeutic Applications of RNAi. Chichester, UK: John Wiley and Sons, Ltd; 2013:417–437. doi:10.1002/9781118610749.ch18

95. Han H, Son S, Son S, et al. Reducible Polyethylenimine Nanoparticles for Efficient siRNA Delivery in Corneal Neovascularization Therapy. Macromol Biosci. 2016;16(11):1583–1597. doi:10.1002/mabi.201600051 [PubMed: 27503770]

Table 1.

Summary of key studies pertaining to epigenetics of the anterior segment

Study (Reference)	Research Category	Location	Tissue type	Study Group	Control group (if applicable)	Conclusion/Significance
DNA methylation and its role in anterior segment disease						
Bonnin 2014 (9)	DNMT expression profiles	France	Human	• 5 normal human corneas from corneal graft bank • 5 conjunctiva and anterior lens capsule samples collected during cataract surgery		• DNMTs are highly expressed in the anterior eye • Suggests that DNA methylation is relevant in ocular tissue
Luo 2019 (19)	Corneal wound healing	China	Mouse	• 8-week-old C57BL/6 mice: right eye debrided with corneal rust ring remover	• 8-week-old C57BL/6 mice: un-traumatized left eye	• During corneal epithelial wound healing, increased expression of DNMT1 and DNMT3B10 cause global hypermethylation • siRNA-mediated silencing of DNMT1 can delay corneal epithelial healing • Identifies DNMT1 and DNA methylation upregulation as novel therapeutic targets to facilitate corneal epithelial wound healing
Khuc 2017 (20)	Fuchs Dystrophy	USA	Human	• Corneal endothelial tissue from 9 patients with FECD, collected during endothelial keratoplasty	• Corneal endothelial tissue from 4 patients without FECD, obtained from eye bank (SightLife and San Diego Eye Bank)	• Impaired fluid transport, cellular homeostasis, and cytoskeletal organization associated with gene methylation levels • DNA methylation patterns play role in loss of corneal transparency in FECD • Establishes DNA methylation patterns as a potential therapeutic target in FECD
Peek 1991 (10)	Lens development	Netherlands	Rat	• DNA isolated from rat lens tissue		• Establishes that de-methylation of the γ-crystallin promoter gene is required for lens epithelial cell differentiation and maintaining refractive index in rats

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Study (Reference)	Research Category	Location	Tissue type	Study Group	Control group (if applicable)	Conclusion/Significance
Klok 1998 (23)	Lens development	Netherlands	Rat	• Lens epithelial explants from newborn and 10-day-old Wistar rats		• Demethylation increases expression of γ-crystallin12
Tittle 2011 (24)	Lens development	USA	Zebrafish	• Transgenic Tg(beta actin2:mCherry-CAAX) zebrafish with mutant alleles uhrf1(hi3020), dnmt1(s872), and dnmt1(s904)		• Zebrafish mutants without DNMT1 exhibited apoptosis and reduced proliferation of lens cells, with subsequent defects in lens development • Confirmed that DNMT1 is required for lens development
Hoang 2017 (11)	Lens development	USA	Mouse	• DNMT1, DNMT3a, and DNMT3b knockout mice produced by breeding loxP-flanked mice with Le-Cre or MLR10-Cre mice		• Inactivation of DNMT1 during embryonic development results in DNA hypomethylation and apoptosis of lens epithelial cells
Li 2013 (25)	Age-related cataracts	China	Human	• 18 lens samples collected from patients with ARC	• 15 clear lens samples collected from patients with rhegmatogenous retinal detachment	• Hypermethylation of MGMT, a DNA repair gene, contributes to development of cataract
Wang 2015 (26)	Age-related cataracts	China	Human	• Lens samples from 15 patients with cortical ARC	• Age-matched patients without ARC, obtained during vitreoretinal surgeries	• Cataract samples had hypermethylation of OGG1, a base excision repair protein • Demethylation of OGG1 protected human lens cells against apoptosis induced by UVB light • Identifies OGG1 as a potential therapeutic target
Wang 2017 (27)	Age-related cataracts	China	Human	• 30 anterior capsules from patients with ARC	• 30 clear lens samples from donors (Eye Bank of Affiliated Hospital of Nantong University)	• Identified five other genes (DNMT3B, HDAC, HDAC4, HDAC9, and MBD3) with increased expression in ARC
McDonnell 2016 (28, 30)	Trabecular meshwork, glaucoma	Ireland	Human	• Normal and glaucomatous trabecular meshwork		• Identified distinct methylation profiles between glaucomatous and healthy cells

Study (Reference)	Research Category	Location	Tissue type	Study Group	Control group (if applicable)	Conclusion/Significance
				cells obtained from Lion's Eye Institute for Transplant and Research		• Glaucomatous TM cells had decreased RASAL1, increased TGF-β1, and an overall increased DNMT1 expression • Hypoxia triggered normal cells to display similar expression and methylation profiles as the glaucomatous TM cells
Matsuda 2015 (32)	Trabecular meshwork, glaucoma	Japan	Human	• Primary culture human trabecular meshwork cells (ScienCell Research Laboratories)		• Treating cultured human TM cells with dexamethasone (to induce glaucoma) led to demethylation of promoter regions for three genes (FKBP5, ZBTB16, SCNN1A) and methylation of promoter regions for 4 genes (ARSI, HIC1, GREM2, and MATN2)
Post-translational histone modification and anterior segment diseases						
Sharma 2016 (47)	Ocular surface fibrosis and wound healing	USA	Rabbit	• Bleb tissues from rabbits which received SAHA (50μM) following glaucoma filtration surgery	• Bleb tissues from rabbits which received balanced salt solution following glaucoma filtration surgery	• Fibrotic postoperative conjunctival healing associated with deacetylation of histones H3 and H4 • SAHA reduced scarring, neovascularization, edema, and corneal opacity, while increasing acetylation of histone H3 and H4 • Highlights promise of using HDACis as anti-fibrotic agents in ophthalmic conditions
Maeng 2015 (38)	Granular corneal dystrophy	South Korea	Human	• 2 human corneal fibroblasts from GCD2-homozygous donors (eye bank, Yonsei University Severance Hospital)	• 2 human corneal fibroblasts without GCD2 mutation	• TGFβ1 led to increased histone methylation at gene promoters which enables transcription factor binding and subsequent upregulated expression of ECM-producing genes • Establishes epigenetic regulation of histone H3K4me in corneal fibroblasts as a potential therapeutic strategy for granular corneal dystrophy

Study (Reference)	Research Category	Location	Tissue type	Study Group	Control group (if applicable)	Conclusion/Significance
Herencia-Bueno 2018 (48)	Diabetes	Brazil	Rat	• 20 rats with diabetes (serum glucose > 400mg/dL), induced by intraperitoneal alloxan injection	• 20 nondiabetic rats (serum glucose < 150mg/dL)	• Diabetes reduced histone H3 acetylation in the cornea, leading to aberrant chromatin organization
Gardner 2013 (49)	Uveitis	United Kingdom	Mouse	• B10.RIII mice inducted with subcutaneous IRBP, mycobacterium tuberculosis complete H37 Ra, and bordetella pertussis toxin		• Up-regulation of deacetylases (e.g. SIRT1) is protective against cataract formation
Wolf 2013 (12)	Lens development	USA	Mouse	• Knockout mice embryos homozygous for CBP and P300 genes	• Mice embryos heterozygous for CBP and P300 genes	• Inactivated of CBP and p300 in embryonic ectodermal lens cells causes aphakia • Shows that histone modifiers such as the acetyltransferases CBP and p300 play a key role in normal cell differentiation
Wang 2016 (36)	Age-related nuclear cataracts (ARNC)	China	Human	• Lens of 30 patients with ARNCs	• Transparent lens of 30 patients who underwent surgery due to vitreoretinal diseases	• UVB exposure caused H3K9 deacetylation and subsequent repression of ERCC6, which codes the CSB protein involved in nucleotide excision repair, leading to cataracts formation • Shows that histone modification may be triggered by UVB light exposure, which represses expression of nucleotide excision repair proteins • Supports an epigenetic mechanism of ARNC formation, as ARNC samples did not share polymorphisms in the ERCC6 gene
Li 2016 (37)	ARNC	China	Human	• 120 ARC patients with cortical, nuclear, or posterior subcapsular cataracts	• 40 patients who had clear lens extraction due to vitreoretinal diseases	• Histone modifications are involved in regulating expression of GSTM3, an antioxidant enzyme protective against ARC • ARNC patients have transcriptional repression of GSTM3 mediated in part by deacetylated histone H3 and

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Study (Reference)	Research Category	Location	Tissue type	Study Group	Control group (if applicable)	Conclusion/Significance
						methylation at histone H3 Lysine 9 (H3K9) • Identifies potential therapeutic target, as transcriptional repression was reversed after treatment with histone deacetylase inhibitor
Ganatra 2015 (34)	Epithelial-mesenchymal transition (EMT) of lens epithelial cells (LECs)	India	Human	• Fetal human LEC line (FHL124) cultures exposed to TGF-β2 (Invitrogen, Carlsbad, CA, USA) and TSA (Sigma-Aldrich)	• Fetal human LEC line (FHL124) cultures exposed to only TGF-β2 (Invitrogen, Carlsbad, CA, USA)	• TGF-β leads to histone acetylation and upregulation of α-SMA, which is active in lens capsule fibrosis and EMT • Trichostatin-A, an inhibitor of HDAC, suppresses EMT by decreasing histone H4 acetylation at the α-SMA promoter, thereby reducing fibrosis
Chromatin remodeling and anterior segment diseases						
Hassan 2014 (54)	Photocarcinogenesis	Australia	Mouse	• Irradiated C57BL/6 mice without expression of BRM protein (Brm−/− and Trp53+/−)	• Irradiated wild-type mice	• BRM knockout mice exhibited more hyperplasia and disorganized proliferation of corneal epithelial/stromal cells • Shows that Brahma (BRM), one of the two ATPase subunits of the SWI/SNF chromatin remodeling complex, is protective against photocarcinogenesis.
Tsui 2016 (55)	Corneal epithelial differentiation	USA	Mouse	• Human telomerase-immortalized corneal epithelial cells (J.V. Jester laboratory), differentiation induced by adding calcium and FBS to culture • Human limbal stem/progenitor (HLS/P) cell culture (sclerocorneal tissues from Illinois Eye Bank, Lions Eye Institute) • Human corneal epithelial cells induced with collagen/	• HTCE cells without CTCF function (after infection with lentiviral particle)	• CTCF-mediated chromatin remodeling may modulate corneal epithelial cell differentiation by acting as a mediator between transcription factor PAX6 and cell differentiation genes

Study (Reference)	Research Category	Location	Tissue type	Study Group	Control group (if applicable)	Conclusion/Significance
				fibronectin-coated surface and airlift procedure		
Dirscherl 2005 (51)	Ocular development	USA	Frog	• Xenopus embryos from in vitro fertilization, injected with antisense ISWI RNA or anti-ISWI morpholino	• Xenopus embryos from in vitro fertilization, injected with water, GFP mRNA, or control morpholino	• Frog embryos injected with dominant-negative ISWI mutant mRNA developed defects in lens development and cataracts • Establishes that ISWI is important for lens development
He 2010 (53)	Lens development	USA	Mouse	• Transgenic mice with dominant-negative Brg1	• Wild-type mice	• Transgenic mice with dysfunctional BRG1 developed cataracts • Shows that BRG1 is important in lens development
He 2016 (13)	Lens development	USA	Mouse	• Transgenic mice with Snf2h−/− and Brg1−/−	• Wild-type mice	• Snf2h (chromatin remodeling enzyme) knockout mice form cataracts and disorganized lens • Shows that Snf2h is necessary for lens development
Non-coding RNA and anterior segment diseases						
Drewry 2016 (14)	miRNA profiles of anterior segment	USA	Human	• 7 ciliary body, 7 cornea, and 7 trabecular meshwork samples from 14 donors (North Carolina Eye Bank) without significant ocular history		• Shows that ocular miRNA is disproportionately expressed, with 11 of the 378 known miRNAs comprising 80% of miRNA present • Identified mutations in miR-184 associated with keratoconus and cataracts • Suggests that miRNA expression plays an essential role in the pathogenesis of ocular diseases such as glaucoma
Ryan 2006 (15)	miRNA profiles of anterior segment	USA	Mouse	• Corneal epithelium and lens from 100 adult CD-1 mice		• Identified the most heavily expressed miRNAs in the lens and cornea (mir-184, mir-204, and mir-205)

Study (Reference)	Research Category	Location	Tissue type	Study Group	Control group (if applicable)	Conclusion/Significance
Lee 2011 (68)	Corneal epithelium	China	Human	• Human corneal rims from adult donors (Joint Shantou International Eye Centre)		• Transfection of corneal epithelial progenitor cells with miR-145 plasmid resulted in a thinner and underdeveloped epithelium • miR-145 likely regulates immune response and promotes differentiation while suppressing proliferation
Teng 2015 (70)	Corneal epithelium	China	Human	• 9 human corneas from adult donors (Joint Shantou International Eye Centre)		• Identified other miRNAs (miR-10b, 126, and 155) that regulate progenitor cell homeostasis by targeting genes encoding transcription factors involved in proliferation and apoptosis, structural proteins such as connexins, and mediators in T cell and B cell receptor signaling
Peng 2012 (68)	Corneal epithelial differentiation	USA	Human	• Corneal epithelial cells from five patients with diabetic keratopathies	• 50 normal human corneas (Midwest Eye Banks, Ann Arbor, MI)	• miR-31 promotes differentiation by indirectly upregulating Notch activity
Peng 2015 (66)	Stem cell niche in corneal and limbal epithelium	USA	Human	• Limbal/corneal epithelia from wild-type mice (BALB/c) obtained from the Charles River Laboratories (Chicago, IL) • Primary cultures of human limbal epithelial keratinocytes and human corneal epithelial keratinocytes from cadaver donor corneas (Midwest Eye Bank) with miR-103 and miR-107 knockdown		• miRs-103 and 107 enhance proliferation via different targets, including kinase p90RSK2, Wnt3a, NEDD9 (HEF1), and tyrosine phosphatase PTPRM48 • miR-450b affects differentiation of limbal cells by repressing the SOX2/P63 pathway
Park 2017 (67)	Corneal angiogenesis	USA	Human, mouse	• Primary human corneal/limbal epithelial keratinocytes from cadaver donor corneas (Midwest Eye	• Primary human corneal/limbal epithelial keratinocytes from cadaver donor	• miR-184 downregulates corneal angiogenesis during wound healing by targeting transcriptional regulators of Akt/VEGF pathway

Study (Reference)	Research Category	Location	Tissue type	Study Group	Control group (if applicable)	Conclusion/Significance
				Bank, Ann Arbor) transfected with pre-miRNA-184 mimetic oligonucleotide • Wild-type C57Bl/6 mice (Charles River Laboratories)	corneas (Midwest Eye Bank, Ann Arbor) transfected with control oligonucleotide	• miR-184 expression in human epithelial cells reduces neovascularization
An 2015 (63)	Corneal wound healing	China	Mouse	• Corneal epithelium from one eye (of female C57BL/6 mice) with corneal wound secondary to corneal rust ring remover	• Corneal epithelium from contralateral eye (of female C57BL/6 mice) without corneal wound	• Corneal wound healing in mice involved changes in expression of 29 miRNAs, especially miR-204 • Transfection of miR-204 into human corneal epithelial cells dramatically reduced cellular proliferation
Mulik 2012 (71)	Neovascularizion in HSV-induced stromal keratitis	USA	Mouse	• Female C57BL/6 mice (Sprague Dawley Inc.), IL-17RKO mice (C57BL/6 background, Amgen) infected with HSV-1 RE Tumpey to one eye and treated with antagomir-132 nanoparticles	• Female C57BL/6 mice (Sprague Dawley Inc.), IL-17RKO mice (C57BL/6 background, Amgen) infected with HSV-1 RE Tumpey to one eye and treated with scrambled sequences of nanoparticles	• Numerous miRNAs are involved in the pathogenesis of HSV keratitis, including miR-132, which is associated with a rise in VEGF and IL-17 • Knockdown of miR-132 reduces corneal angiogenesis and inflammation
Bhela 2015 (72)	HSV-induced stromal keratitis	USA	Mouse	• Female C57BL/6 mice (Sprague Dawley Inc.), Mir155−/− mice (C57BL/6 background, The Jackson Laboratory) infected with HSV-1 RE Tumpey to one eye and injected with subconjunctival antagomir-155	• Female C57BL/6 mice (Sprague Dawley Inc.), Mir155−/− mice (C57BL/6 background, The Jackson Laboratory) infected with HSV-1 RE Tumpey to one eye and injected with subconjunctival scrambled sequence	• Silencing MiR-155 attenuates T helper cell response, neovascularization, severity of lesion
Kulkarni 2017 (73)	Diabetes	USA	Human	• RNA extracted from 12 diabetic cadaver corneas (National Disease Research Interchange, NDRI)	• RNA extracted from 10 normal cadaver corneas (National Disease Research Interchange, NDRI)	• Diabetes upregulates miR-10b, which increases corneal epithelial proliferation

Study (Reference)	Research Category	Location	Tissue type	Study Group	Control group (if applicable)	Conclusion/Significance
				• Human corneal epithelial cells transfected with hsa-miR-10b-5p mimic or inhibitor	• Human corneal epithelial cells transfected with negative controls	
Winkler 2014 (74)	Diabetes, wound healing	USA	Human	• 14 diabetic human cadaver corneas (National Disease Research Interchange) • 8 pairs of diabetic organ-cultured corneas (one cornea in each pair transfected with miR-146a mimic or miR-146a inhibitor)	• 11 normal human cadaver corneas (National Disease Research Interchange) • 8 pairs of diabetic organ-cultured corneas (other cornea in each pair transfected with control Cy3-labeled scrambled sequence miRNA)	• Diabetes upregulates miR-146a, which impairs wound healing and migration
Chen 2017 (76)	Lens fibrosis	China	Human, mouse	• Human LEC line SRA01/04 (Tufts University) treated with TGFβ2, DAPT (Notch receptor cleavage inhibitor), and SB431542 (TGFB signaling inhibitor) • Mice divided into 3 groups of 15 each (miR-26a, miR-26b, and negative control agomirs injected into wounded eyes) receiving DAPT	• Human LEC line SRA01/04 (Tufts University) treated with TGFβ2, DAPT (Notch receptor cleavage inhibitor), and SB431542 (TGFB signaling inhibitor) • Mice divided into 3 groups of 15 each (negative control agomirs injected into wounded eyes) receiving DMSO as control	• miR-26a and -26b directly target Jagged-1 and suppress Jagged-1/Notch signaling • EMT and lens fibrosis could be reversed in lens epithelial cells
Yu-Wai-Man 2016 (78)	Lens fibrosis	United Kingdom	Human	• Primary human Tenon's fibroblasts isolated from donor eyes (eye bank), transfected with MRTF-B siRNAs	• Primary human Tenon's fibroblasts isolated from donor eyes (eye bank), transfected with negative control siRNA	• Receptor-targeted liposome-peptide-siRNA nanoparticles can effectively deliver siRNA and silence the MRTF-B gene by up to 76% • Establishes feasibility of siRNA targeting in therapeutic strategies in ocular disease
Zhang 2016 (79)	Posterior capsule opacification (PCO)	China	Human	• Human lens epithelial cells (American Type	• Human lens epithelial cells	• Human LECs that overexpressed MiR-486–5p had

Study (Reference)	Research Category	Location	Tissue type	Study Group	Control group (if applicable)	Conclusion/Significance
				Culture Collection) transfected with MTOR-siRNA	(American Type Culture Collection) mock transfected with reagent	reduced wound healing, proliferation, EMT, and migration
Liu 2017 (80)	PCO	China	Human	• Human lens epithelial cells (American Type Culture Collection) transfected with MiR-486–5p mimic and/or Smad2 plasmids	• Human lens epithelial cells (American Type Culture Collection) transfected with miR-NC mimics or empty plasmids	• Transfection of siRNA of mTOR inhibits mTOR/ p70S6K/Akt and suppressed expression of mTORC1, mTORC2, and TGF-β-induced EMT • Identifies mTOR, p70S6K, and PKB as potential therapeutic targets in PCO