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Review

N6-Methyladenosine-Modification-Related Ophthalmic Diseases and Potential Therapeutic Strategies

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Abstract

The N6-methyladenosine (m6A) is the most abundant internal modification in advanced eukaryotic mRNAs, and it plays an important role in mRNA metabolism and diverse biological processes. Moreover, m6A modification is dynamically reversible and may reshape gene expression patterns after demethylation induced by drug interventions, which may reverse the occurrence and progression of certain diseases. Although the role of changes in DNA methylation in ophthalmic diseases has been well described, the regulatory role of the m6A modification in ophthalmic diseases is still a new field of study. This paper aims to systematically summarize the latest research progress about m6a-modification-related ophthalmic diseases and potential therapeutic strategies. All English literature relevant to our research was searched in PubMed and CNKI databases, using appropriate keywords. Our study reviews the regulatory role of m6A in ophthalmic diseases. It covers almost all of the reported m6A-related ophthalmic diseases and proposes potential treatment strategies for each disease. This review will provide direction for further research on m6A in ophthalmic diseases and help in the treatment of ophthalmic diseases in the future.

Keywords: m6A modification; m6A enzymes; ophthalmic diseases; potential therapeutic strategies

1. m6A Regulators

1.1 m6A

Epigenetic modifications, including chemical modifications of DNA, RNA, and proteins, result in altered gene expression levels and function, without changing the nucleotide or amino acid sequence. In recent years, based on well-established epigenetic modifications of DNA and proteins, research on reversible RNA methylation has led to a third wave in the epigenetic field. There are more than 100 known chemical modifications of RNA, but methylation is the most important modification. The N6-methyladenosine (m6A) modification is one of the most prevalent RNA modifications in eukaryotes. It modifies the base sites on RNA, thus affecting the translation, degradation, and splicing of RNA. As early as the 1970s, Desrosiers *et al.* [1] detected the m6A modification in mRNA, but it was not until 2011 that the conserved sequence, *RRACH* (R indicates A or G and H indicates A, U, or C), was identified as a selective site for the m6A modification in mRNA [2]. The discovery of the demethylase enzyme, fat mass and obesity-associated protein (FTO), in 2012 triggered a large amount of innovative scientific inquiry [3]. The m6A modification is mainly regulated by three homologous factors, namely the so-called "writer", "eraser", and "reader".

1.2 m6A Writers

m6A writers contribute to the formation of the methyltransferase complex, which catalyzes the formation of m6A. Methyltransferase-like 3 (METTL3) is an important component of this complex and it mainly acts as the catalytic core. Methyltransferase like 14 (METTL14), another active component of the complex, acts as an RNA-binding platform and is a significant component for the promotion of RNA binding [4]. Wilms' tumor 1-associating protein (WTAP) acts as a regulatory subunit and regulates the location and binding of the m6A catalytic methyltransferase METTL3/METTL14 complex to mRNA [5]. The homolog of the METTL3 protein, methyltransferase like 16 (METTL16), regulates intracellular S-adenosyl methionine levels by dynamically regulating the m6A modification of the small nuclear RNA, U6, and target mRNAs [6]. The m6A methyltransferase complex is a multifactorial functional complex. In addition to the core components mentioned above, the complex includes multiple regulatory subunits, such as vir-like m6A methyltransferase-associated (VIRMA, also known as KIAA1429) [7], RNA binding motif protein 15 (RBM15/15B) [8], and zinc finger CCCH-type-containing 13 (ZC3H13) [9]. VIRMA recruits the catalytic core component METTL3/METTL14/WTAP to guide regioselective methylation. RBM15/15B consistently methylates adenotides in adjacent m6A residues by binding to the m6A-methylation complex and recruiting it to RNA molecules. ZC3H13 anchors WTAP, a virilizer protein, and Hakai to the nucleus to promote m6A methylation. For Methyltransferase like 5 (METTL5), it can catalyze m6A on certain structural RNA, including 18S rRNA,

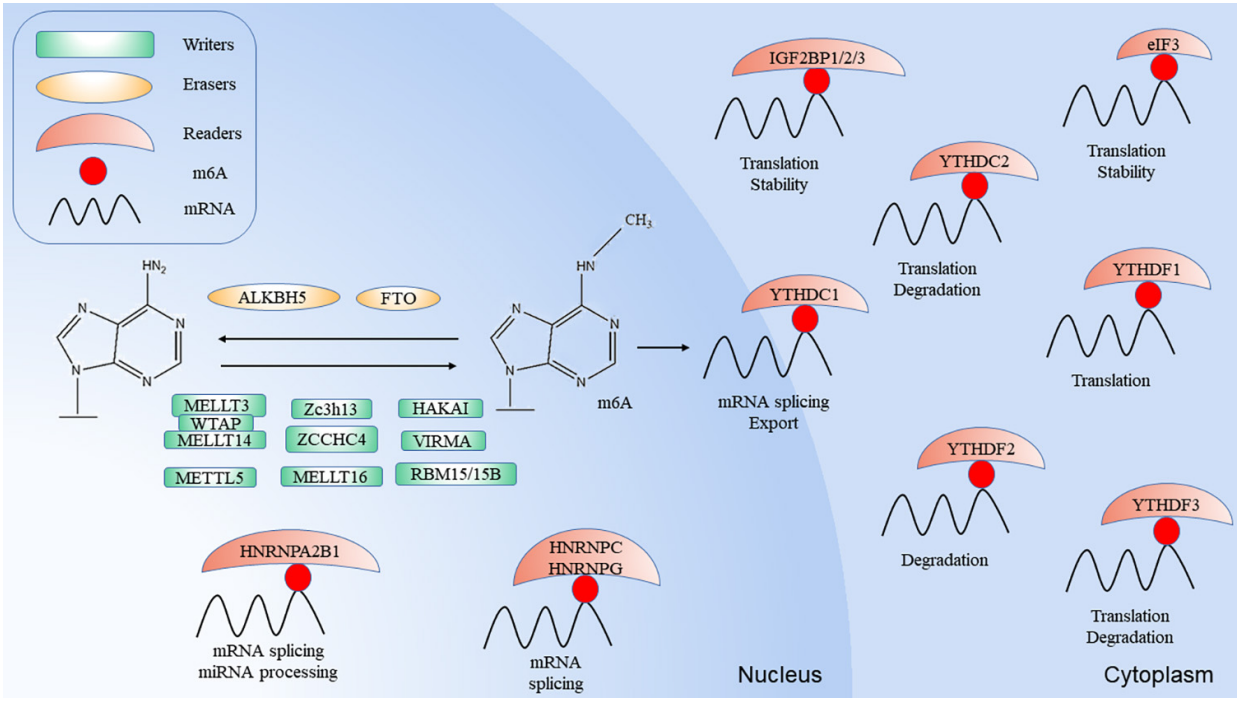

Fig. 1. The m6A modification. The m6A modification is dynamically regulated by "writers" (METTL3, METTL14, WTAP, METTL16, VIRMA, RBM15/15B, ZC3H13, METTL5, ZCCHC4, HAKAI) and "erasers" (FTO and ALKBH5). "readers" (YTHDF1, YTHDF2, YTHFDF3, YTHDC1, YTHDC2, HNRNPA2B1, HNRNPC/HNRNPG, IGF2BP1/2/3 and eIF3) are binding proteins of m6A. Different regulators of m6A have different functions on transcriptional process.

28SrRNA, and U6 small nuclear (snRNA) [10]. ZCCHC4-mediated rRNA m6A methylation affects the distribution of ribosome subunit, translation, and cell proliferation, which can lead to tumorigenesis [11]. HAKAI, also known as Cbl Proto-Oncogene Like 1 (CBLL1), serves primarily to assist in the control of nuclear m6A methylation [12].

1.3 m6A Erasers

The discovery of m6A erasers revealed, for the first time, the reversibility of RNA chemical modification and established the theoretical foundation for a new field of transcriptomic research on RNA methylation. Jia *et al.* [3] confirmed the FTO-catalyzed demethylation of m6A modifications of mRNA by measuring m6A levels in FTO-gene-knockdown versus FTO-overexpressing cells. AlkB homolog 5 (ALKBH5), a member of the AlkB family, is another mammalian demethylase that can reverse m6A in mRNA *in vivo* and *in vitro*. This demethylation activity of ALKBH5 can affect not only mRNA export and RNA metabolism, but also the assembly of mRNA processing factors in nuclear spots [13].

1.4 m6A Readers

The m6A readers can recognize and bind to the m6A-modified transcript regulating gene expression through regulating diverse processes, such as mRNA stability, mRNA splicing, mRNA structure, mRNA export, translation efficiency and miRNA biogenesis [14]. The main readers are members of the YTHD protein family, which recognize m6A modifications and regulate mRNA fate [15]. YTH domain-containing 1 (YTHDC1) recruits serine and arginine-rich splicing factor 3 (SRSF3) to RNA binding sites and simultaneously suppresses SRSF10 at the RNA binding site. This suggests that the m6A modification affects transcripts in the nucleus by regulating alternative splicing [16] and interactions between SRSF3 and nuclear RNA export factor 1 (NXF1) to promote the nuclear export of m6A-modified mRNA [17]. YTHDC2 enhances translation efficiency or decreases the abundance of its substrates [18]. YTH domain family member 1 (YTHDF1) acts directly on the translation initiation complex, thereby promoting the translational efficiency of m6A-modified RNA [19]. YTHDF2 accelerates the degradation of m6A-modified transcripts by recruiting the C-C chemokine receptor type 4-NOT deadenylation complex [20]. In addition, it can regulates the stability of mRNA. The YTHDF3 and YTHDF1 binding motifs are similar and they jointly regulate mRNA translation efficiency [21]; moreover, YTHDF3 also mediates mRNA degradation by directly interacting with YTHDF2 [22].

Recently, the heterogeneous nuclear ribonucleoprotein (hnRNP) family, insulin-like growth factor 2 mRNA-binding protein (IGF2BP) family, and initiation factor 3 (eIF3) have been shown to be m6A-binding proteins [23]. hnRNPA2B1 promotes alternative splicing by recruiting the microprocessor complex to bind to m6A and mediate

microRNA (miRNA) processing, while hnRNPC and hnRNPG mediate the alternative splicing of m6a-modified transcripts. eIF3 binds to the m6A site in the 5′-untranslated region (UTR) of mRNA, acting together with IGF2BP1, 2, and 3 to stabilize the target gene and initiate the translation process (Fig. 1, Table 1).

1.5 m6A-Related Diseases

Recent studies have shown that the m6A modification is closely related to many biological functions, such as the regulation epithelial-mesenchymal transition (EMT), the DNA damage response, gene translation, autophagy, adipogenesis, and the determination of stem cell fate [24]. In addition, the correlation between m6A modification and autoimmune responses is gaining prominence, and there is growing evidence that the m6A pathway contributes to the progression of certain autoinflammatory diseases [25]. Specifically, expression of certain m6A enzymes (ALKBH5 and YTHDF2) are decreased in peripheral blood mononuclear cells from patients with systemic lupus erythematosus or rheumatoid arthritis [26,27]. *IL6* and *Lcn2* (encoding Lipocalin-2) mRNAs are strongly dependent on IGF2BP2, both genes have been identified as important drivers of autoimmune inflammation in autoantibody-induced glomerulonephritis in the IL-17-driven immune environment [28]. M6A also plays an important role in regulating many aspects of the antiviral response. For example, during vesicular stomatitis virus infection, the siRNA consumption of METTL3 increases the virus's dsRNA, resulting in more RIG-I binding to viral RNA, worsening the infection [29]. In addition, numerous studies on the oncogenic mechanism of m6A were summarized by Gu *et al*. [30]. FTO enhances leukemia oncogene-mediated cell transformation and leukemia, inhibits all trans retinoic aci–mediated AML cell differentiation, and regulates mRNA synthesis of target genes such as *ASB2* and *RARA* by down-regulating m6A levels [31]. ALKBH5 induces demethylation of the transcription factor FOXM and stimulates cell proliferation. METTL3 promotes tumor growth by targeting the 30 UTR of SOX2 mRNA [32,33]. METTL3 is an oncogene of lung cancer, which plays a carcinogenic role through different mechanisms, such as recruiting translation initiation factors, increasing EGFR, regulating some miRNAs, etc. [34]. FTO promotes the proliferation of non-small cell lung cancer by increasing the expression of ubiquitin-specific protease 7 [35]. The deletion of METTL14 or overexpression of FTO can increase endometrial cancer cell proliferation, clone formation, and metastasis [36,37]. There are also Cervical Cancer, Ovarian Cancer, Breast Cancer, etc., and these mechanisms provide new strategies for drug development and clinical cancer therapy. The role of m6A in some diseases has been well elucidated, but the regulatory role of m6A in ophthalmic diseases is still a new field of study. Here, we review the latest research progress on m6A modification-related ophthalmic diseases and propose potential therapeutic strategies. This review will provide direction for further research on m6A in ophthalmic diseases and contribute to the development of new treatments for ophthalmic diseases.

2. m6A-Modification-Related Ophthalmic Diseases

2.1 The m6A Modification in Pterygia

A pterygium is a triangular piece of fibrovascular tissue that grows from the bulbar conjunctiva of the palpebral fissure to the cornea and often occurs on the side of the nose. It is a common ocular surface disease and is generally believed to be a chronic inflammatory lesion caused by external stimuli. The incidence of pterygia in Asia is 7%, and the recurrence rate after surgical resection is high [38]. Therefore, it is necessary to explore the specific mechanisms of pterygiogenesis. Recently, investigators examined the roles of DNA [39] and EMT [40] in the pathogenesis of pterygia. The involvement of the m6A modification in biological functions, such as regulating EMT and the DNA damage response, suggests a possible mechanism for the involvement of this modification in the development of pterygia.

Jiang *et al*. [41] performed a series of experiments to show the distinct downregulation of m6A levels in pterygia compared to normal conjunctival tissues. The pterygium tissue is obtained by surgical excision and a small piece of normal conjunctival tissue is taken from the contralateral corneal margin of the same eye as a control. An analysis of the mRNA expression levels of five key enzymes (METTL3, METTL14, WTAP, FTO, and ALKBH5) required for the m6a modification showed that the mRNA levels of METTL3 were abnormally low in pterygia compared to conjunctiva. Western blotting analysis revealed a similar decrease in METTL3 protein expression levels. These results suggest that METTL3 is a crucial enzyme in the development of pterygia. In addition, by analyzing differential m6A methylation peaks and concurrently differentially expressed genes, Jiang *et al*. [41] identified five genes (Desmoplakin [*DSP*], Recombinant Matrix Remodelling Associated Protein 5 [MXRA5], Rho GTPase Activating Protein 35 [ARHGAP35], Transmembrane protein 43 [TMEM43], and Olfactomedin-like2A [OLFML2A]) related to the development of pterygia, indicating a potential connection between m6A methylation and gene expression levels. *DSP* has different roles in cancer; however, it is generally known to be a key factor in EMT [42]. *MXRA5* encodes a matrix remodeling-associated protein, and its high expression level in pterygia suggests that it reconstructs the outer matrix by activating transforming growth factor-β (TGF-β) [43]. *ARHGAP35* increases cell invasion and migration and induces or promotes the development of pterygia [44]. *TMEM43* plays an important role in maintaining the nuclear membrane structure, and its high expression level in pterygia may be important for the control of cell survival, migration, and invasion via the epidermal growth fac-

Table 1. Characteristics and functions of m6A enzymes in m6A modifications.

Category	Enzyme	Characteristics and functions	Related ophthalmic diseases
Writers	METTL3	Catalytic subunit, acting as the catalytic core	Pterygium High myopia Glaucoma Traumatic optic neuropathy Diabetic cataract Diabetic retinopathy Proliferative vitreoretinopathy Ocular melanomas Retinoblastoma
	METTL14	Catalytic subunit, acts as an RNA-binding platform and promotes RNA binding	High myopia Age-related cataract Diabetic retinopathy
	WTAP	Regulatory subunit, regulates METTL3/METTL14 complex locates and binds mRNA	Graves' ophthalmopathy Traumatic optic neuropathy Diabetic retinopathy
	METTL16	Regulatory subunit, regulates intracellular S-adenosyl methionine levels by dynamically regulating the m6A modification of the small nuclear RNA, U6, and target mRNAs	/
	VIRMA	Regulatory subunit, recruits the catalytic core component, METTL3/METTL14/WTAP to guide regioselective methylation	/
	RBM15/15B	Regulatory subunit, consistently methylates adenotides in adjacent m6A residues by binding to the m6A-methylation complex and recruiting it to RNA molecules	Ocular melanomas
	Zc3h13	Regulatory subunit, anchors WTAP, a virilizer protein, and Hakai to the nucleus to promote m6A methylation	/
	METTL5	Catalytic subunit, catalyzes m6A on some certain structured RNAs	/
	ZCCHC4	Regulatory subunit, makes a difference to the distribution of ribosome subunit, global translation, and cell proliferation	/
	HAKAI	Regulatory subunit, assists controlling nuclear m6A methylation	/
Erasers	FTO	Catalyze the demethylation of m6A modifications on the mRNA	High myopia Traumatic optic neuropathy Diabetic retinopathy
	ALKBH5	Catalyze the demethylation of m6A modifications on the mRNA	High myopia Traumatic optic neuropathy Age-related cataract Diabetic retinopathy Ocular melanomas
Readers	YTHDC1	Regulates the mode of alternative splicing; promotes the nuclear exit of m6A-modified mRNA	/
	YTHDC2	Enhance the translation efficiency or decrease the abundance of its substrate	Graves' ophthalmopathy
	YTHDF1	Promote the translation efficiency of m6A-modified RNA substrates	High myopia
	YTHDF2	Accelerating the degradation of the m6A-modified transcripts; regulation of the mRNA stability	High myopia Graves' ophthalmopathy Glaucoma Diabetic retinopathy
	YTHDF3	Cooperate with YTHDF1 to regulate mRNA translation efficiency; mediate mRNA degradation	/
	HNRNPA2B1	Promotes alternative splicing by recruiting the microprocessor complex binds to m6A for mediating the processing of miRNA	/
	HNRNPC/HNRNPG	Mediates alternative splicing of m6a-modified transcripts	/
	IGF2BP1/2/3	Cooperate with eIF3 to stabilize target genes and initiate the translation process	/
	eIF3	Cooperate with IGF2BP1/2/3 to stabilize target genes and initiate the translation process	/

tor receptor signaling pathway [45]. *OLFML2A* is a novel TGF-β regulator that induces smooth muscle differentiation and is a novel oncogenic factor in hepatocellular carcinoma. However, its role in pterygia has not yet been elucidated [46].

In summary, the reduced levels of m6A caused by the downregulation of METTL3 may be significant in the development of pterygia, but the exact mechanism should be further investigated. Many genes that were either hypermethylated or hypomethylated were found to be differentially expressed, and therefore, these genes may play important roles in the development of pterygia. However, the association between m6A methylation and gene expression levels remains unclear. The present evidence shows that the upregulation of METTL3 may increase m6A levels and change the expression levels of relevant genes, thus reducing the occurrence of pterygia.

2.2 The m6A Modification in High Myopia

Currently, 163 million people, which is 2.7% of the world's total population, have high myopia, and this number is increasing rapidly [47]. High myopia, defined as a degree of myopia exceeding 6.00 D or an axis length of 26 mm, is a disease that affects almost the entire human eye, from the anterior to the posterior pole. It is characterized by high refractive error and an increased risk of cataracts, open-angle glaucoma, and retinopathy. Excessive axial elongation in patients with high myopia may cause mechanical stretching of the outer layer of the eyeball, leading to various pathological changes such as uveoma, chorioretinal atrophy lesions, patent membrane cracks, and choroidal neovascularization [48].

The cataracts caused by high myopia are mostly nuclear cataracts. To explore the pathogenic mechanism of high myopia, Wen *et al*. [49] detected differences in m6A and gene expression levels between the anterior lens capsule in nuclear cataract patients with and without high myopia. The results showed the upregulation of METTL14 levels and the downregulation of METTL3, FTO, and ALKBH5 levels in the anterior capsule of the lenses of patients with high myopia. These changes disrupted the dynamic balance of m6A modification levels. Moreover, YTHDF1 and YTHDF2 levels were found to be downregulated in high myopic nuclear cataract patients, suggesting that these two readers may participate in the development of high myopia via the post-transcriptional regulation of gene expression.

Gene Ontology analysis showed that hypermethylated genes encoding m6A-containing mRNAs were mainly involved in the formation of the extracellular matrix. Thus, Wen *et al*. [49] proposed that the upregulation of METTL14, and the downregulation of FTO and ALKBH5 induce the hypermethylation of the *CHI3L1* gene, which may affect the expression level of its encoded protein, YKL-40, and promote the pathological state of high myopia by regulating the composition of the extracellular matrix. Posterior scleral staphyloma is one of the most common anatomical changes associated with a series of related degenerative changes. The high expression levels of m6A-modified mRNA may participate in the formation of posterior scleral staphyloma by affecting the extracellular matrix components [50]. In addition, hypermethylated genes encoding m6A-containing mRNAs are also associated with vascular development, suggesting that increased m6A levels may damage the fundus by affecting choroidal circulation.

The evidences presented here provides a solid basis for determining the potential functional role of the m6A modification in pathological ocular damage caused by high myopia. However, the specific mechanism linking m6A levels and pathological ocular damage is not clear. Downregulating the expression of METTL14 and upregulating the expression of METTL3, FTO, and ALKBH5 to reduce m6A levels may be potential research directions for the prevention and treatment of the complications of high myopia.

2.3 The m6A Modification and Graves' Ophthalmopathy

Graves' ophthalmopathy is the most significant extrathyroid manifestation in patients with Graves' disease, and is widely recognized as an autoimmune process. The disease is more common in women, with an annual incidence of 16 women and 3 men per 100,000 people [51]. The clinical manifestations of Graves' ophthalmopathy mainly originate from immune and inflammatory responses in the orbit. This pathological process is thought to be driven by cellular and humoral immunity and inflammation, which stimulate retroocular fibroblast proliferation, local adipogenesis, and inflammatory responses in the extraocular muscle and interstitial tissues. Graves' ophthalmopathy can lead to ocular motility disorders, eyelid retraction, exposed keratopathy, optic nerve compression, and vision loss [52]. Previous studies [53] have shown that immune and inflammatory responses are tightly regulated by m6A methylation. However, the role of m6A methylation status and the underlying mechanism in the pathogenesis of Graves' ophthalmopathy remain unclear.

Zhu *et al*. [54] analyzed the levels of m6A and its associated enzymes in extraocular muscles surgically removed from patients with Graves' ophthalmopathy and exotropia and assessed the correlation of these levels with functional enrichment. The results showed significantly increased m6A levels in Graves' ophthalmopathy tissues compared to tissues from exotropia patients. The polymerase chain reaction (PCR) results were consistent with the RNA sequencing results, which showed that WTAP, YTHDF2, and YTHDC2, but not ALKBH5 and hnRNPA2B1, were significantly upregulated in Graves' ophthalmopathy samples. Gene Ontology enrichment analysis showed that the 10 most upregulated mRNAs had functional involvement in both immune and inflammatory responses, including lym-

phocyte activation, leukocyte differentiation, cytokine production, cytokine-mediated signaling pathways, and adaptive immune responses. Expression analysis of specific markers of inflammation showed that the expression levels of interleukin (IL)-1, IL-6, IL-8, IL-10, IL-17, interferon-γ, and tissue necrosis factor-α were significantly upregulated in the extraocular muscles from patients with Graves' ophthalmopathy. More importantly, an analysis of biological pathways showed that 12 of the 19 pathways implicated in the pathogenesis of Graves' ophthalmopathy were associated with immune and inflammatory responses.

In conclusion, inflammation is a major event in the pathogenesis of Graves' ophthalmopathy. The total m6A levels are significantly increased, and WTAP, YTHDF2, and YTHDC2 levels are significantly upregulated in patients with Graves' ophthalmopathy. However, no further studies have reported a specific association between m6A levels and inflammation. Regulating WTAP, YTHDF2, and YTHDC2 levels to decrease m6a levels may reduce inflammation and delay the development of Graves' ophthalmopathy.

2.4 The m6A Modification and Glaucoma

Glaucoma is the leading cause of irreversible blindness worldwide. The prevalence of glaucoma in people aged 40 to 80 years is currently estimated at 3.5% globally, but as the population ages, 111.8 million people are expected to have glaucoma by 2040 [55]. Glaucoma is a group of progressive optic neuropathies characterized by further deepening and enlargement of the optic cup, apoptotic degeneration of retinal ganglion cells, and a corresponding decline in visual acuity. Ganglion cells transmit visual information to the brain through axons that constitute the optic nerve. The optic disc is the site of axon engagement of retinal ganglion cells, and the optic cup is a depression in the center of the optic disc. In glaucoma, progressive enlargement of the optic cup occurs due to damage to the superficial retina and the loss of axons in retinal ganglion cells [56]. Therefore, precise control of the growth and maintenance of the dendritic structure of ganglion cells is crucial for normal visual function in mammals.

Niu *et al*. [57] found that the m6A reader, YTHDF2, is highly expressed in mouse retinal ganglion cells. Compared with control mice, those with conditional knockout (cKO) of YTHDF2 in the retina had an increased number of retinal ganglion cell dendritic branches, resulting in more synapses in the inner plexiform layer and improved visual acuity. Furthermore, an experimental acute glaucoma model of mice further demonstrated that YTHDF2 cKO in the retina protects ganglion cells from dendritic degeneration. The study of Niu *et al*. [57] identified the m6A-modified YTHDF2 target transcripts that mediate these effects and revealed the mechanism by which YTHDF2 limits retinal ganglion cell dendritic development and maintenance. Available research evidence suggests that the application of YTHDF2 cKO in patients with acute glaucoma may protect their vision. However, this requires further clinical research.

Glaucoma filtration surgery is a classic surgical technique to treat glaucoma, and excessive activation of human Tenon's fibroblasts, which causes scarring, is a cause of surgical failure. However, the mechanism underlying the hyperactivation of human Tenon's fibroblasts is largely unknown. Liu *et al*. [58] first isolated and identified human Tenon's fibroblasts and found that transforming growth factor-1 (TGF-1) enhanced their viability and promoted their proliferation and extracellular matrix deposition. These effects were inhibited by inhibiting METTL3. They used a rabbit model to demonstrate that the METTL3/SMAD3 regulatory axis is abnormally expressed during glaucoma filtration surgery. Subsequently, it was illustrated that increased METTL3 levels played a role in promoting SMAD3 in TGF-β1-induced human Tenon's fibroblasts. However, there is a lack of animal experiments on METTL3/SMAD3 inhibition to confirm that it attenuates scar formation in glaucoma. This provides a new theoretical strategy whereby repressing METTL3/SMAD3 reduces the viability of human Tenon's fibroblasts and inhibits cell proliferation and extracellular matrix deposition by inhibiting TGF-1.

In brief, the implementation of YTHDF2 cKO in patients with acute glaucoma may protect their vision. Besides, repressing METTL3/SMAD3 can inhibit cell proliferation and extracellular matrix deposition by inhibiting TGF-1, thereby reduce the failure of glaucoma filtration surgery.

2.5 The m6A Modification and Traumatic Optic Neuropathy

Traumatic optic neuropathy (TON) is a common complication of traumatic brain injury (TBI) with an incidence of about 1.5–4% [59]. Most patients with TON are visually impaired or even disabled, and their main treatment is corticosteroids and optic nerve decompression surgery, but there are no prospective randomized controlled trials to evaluate the effects of steroids and optic nerve decompression surgery on TON [60]. Recently, it has been found that m6A regulators are expressed differently after TBI, suggesting their involvement in TBI damage [61]. Further, the m6A signaling pathway has been shown to play an important role in neuronal development, and METTL3-mediated m6A modifications may participate in cerebellar development by modulating the stability of the associated mRNA [62].

To explore the relationship between m6A modification and TON, Qu *et al*. [63] detected the expression of m6A-related genes via quantitative real-time PCR (qRT-PCR) and performed methylated RNA immunoprecipitation sequencing (MeRIP-seq) as well as RNA-sequencing to analyze the alteration profiles of m6A modification af-

ter TON. The TON model was established by clamping the optic nerve of rats. The results showed that the expression of m6a-related enzymes (METTL3, WTAP, FTO, ALKBH5) was upregulated after TON. The MeRIP-seq results then showed that 2810 m6A peaks were upregulated and 689 m6A peaks were downregulated. Kyoto Encyclopedia of Genes and Genomes analysis showed that upregulated m6A peaks were significantly correlated with MAPK signaling pathway, NF-κB signaling pathway, and TNF signaling pathway. The downregulated m6A peaks were remarkly correlated with ribosome pathway. The Sarm1-MAPK pathway facilitates the progression of axonal injury by interfering with the energy balance of the axons, causing adenosine triphosphate (ATP) to deplete before complete axon injury [64]. The NF-κB pathway is a key pathway of inflammation in central nervous system injury, which regulates the expression of pro-inflammatory and pro-apoptosis genes in its active form [65]. Regulating MAPK and NF-κB signaling pathways, thereby regulating inflammatory responses, may enable neuroprotective effects [66].

Taken together, expression of methyltransferases and demethylases (METTL3, WTAP, FTO, and ALKBH5) are both up-regulated after TON. These enzymes may work together to raise m6A levels and thereby promote the progression of optic nerve damage through MAPK and NF-κB signaling pathways. This may provide novel insights into the mechanism and treatment of TON.

2.6 The m6A Modification and Cataracts

2.6.1 Age-Related Cataracts

Age-related cataracts (ARCs) are one of the leading causes of visual impairment, accounting for the majority of age-related blindness cases globally. There are three main types of clinically recognizable ARCs: cortical, karyotic, and subcapsular. As the Chinese population ages, the prevalence of cataracts is expected to increase accordingly in the coming years. It is expected that number of cataract patients aged 45–89 years will more than double nationwide by 2050, reaching a prevalence of 33.34% [67]. The key pathways in ARC pathogenesis are genetic and epigenetic regulation. Previous studies [68] have indicated that circular RNAs (circRNAs) regulate the expression of genes in cataracts via miRNAs. Homeodomain-interacting protein kinase 3 circRNA (circHIPK3) regulates the proliferation and apoptosis of human lens epithelial cells through the circHIPK3/miR-193a/alpha-crystallin A chain pathway.

Through a genome-wide analysis of lens epithelial cells, Li *et al*. [69] found that circular RNA levels were lower in ARC patients than in controls; ALKBH5 and METTL14 mRNA levels were higher in ARC patients than in controls; while FTO, METTL3, and WTAP levels were not different between groups. During ARC onset, ultraviolet-B (UV-B) irradiation of the lens causes DNA damage and oxidative stress. A model of oxidative damage in SRA01/04 cells (human lens epithelial cell line) under UV-B irradiation was established, and the experimental cells were analyzed by immunofluorescence after UV-B irradiation. The results showed that ALKBH5 was upregulated in experimental cells. Furthermore, Li *et al*. [69] reported elevated ALKBH5 protein levels in cells after radiation. These results indicate that ALKBH5 may be involved in the process of decreased m6A-modified circRNA expression levels.

In conclusion, circRNA levels are decreased, while ALKBH5 and METTL14 levels are increased in human lens epithelial cells from ARC patients, and radiation increases ALKBH5 levels. Although the specific mechanism responsible for the effect of radiation on ALKBH5 remains unclear, we can infer that decreasing the amount of radiation or downregulating ALKBH5 to increase the expression of m6A-modified circRNAs may decrease the apoptosis of lens epithelial cells, thus delaying the development of ARC.

2.6.2 Diabetic Cataracts

Diabetic cataracts (DCs) are characterized by nubeculae and other disorders of human lens epithelial cells caused by abnormal peripheral blood circulation in diabetic patients [70]. Before the age of 65, people with diabetes have a 4-fold relative risk of developing cataracts than nondiabetic individuals, with poor glycemic control and prolonged disease duration as the main triggers. And patients with type 1 and type 2 diabetes had a 10-year risk of cataract formation at 8.3% and 24.8%, respectively [71]. In the pathogenesis of DC, high glucose levels may cause lens cell apoptosis and metabolic disorders.

Yang *et al*. [72] examined the m6A modification spectrum of human lens epithelial cells under high-glucose and normal-glucose conditions using MeRIP-seq analysis. The results showed that METTL3 was upregulated in DC tissue specimens and high glucose-induced human lens epithelial cells and that the total m6A modification levels were higher in high-glucose-treated human lens epithelial cells than those treated with normal glucose levels. Moreover, a METTL3-specific short hairpin RNA (shRNA) was constructed and transfected into human lens epithelial cells to silence METTL3 mRNA. The resulting downregulation of METTL3 promoted the proliferation of high-glucose-treated human lens epithelial cells. Thus, METTL3 regulates the high-glucose-induced proliferation and apoptosis of human lens epithelial cells. However, compared to normal glucose conditions, western blotting results showed that high glucose conditions increased intercellular adhesion factor-1 (ICAM-1) protein expression levels, while silencing METTL3 inhibited ICAM-1 protein expression levels. RNA stability analysis showed that the mRNA half-life was reduced in cells with METTL3 silenced compared to control cells. Together, these data indicate METTL3 may target the 3′-UTR of ICAM-1 to stabilize its protein expression levels.

High glucose levels induce the upregulation of METTL3 and increase the total m6A levels. METTL3 may then target and stabilize ICAM-1 mRNA, while strongly inhibiting the proliferation of human lens epithelial cells and promoting their apoptosis. It is a pity that these findings are still limited to studies at the cellular level. However, we can infer from these findings that the repression of METTL3 reduces the stability of ICAM-1, inhibits the apoptosis of human lens epithelial cells, and delays the development of DC.

2.7 The m6A Modification and Diabetic Retinopathy

Diabetic retinopathy (DR) is one of the most common microvascular complications associated with diabetes. The incidence varies between regions and the annual incidence of diabetic retinopathy ranged from 2.2% to 12.7% [73]. Retinal microvascular leakage and obstruction caused by chronic progressive diabetes cause a series of fundus lesions, such as microaneurysms, hard exudates, cotton-wool patches, neovascularization, vitreous proliferation, macular edema, and even retinal detachment. The main pathogenic factors associated with DR are inflammation, oxidative stress, angiogenesis, hyperglycemia, and dyslipidemia.

Angiogenesis is one of the most critical factors controlling the progression and pathogenesis of DR and it is also the main target of current treatments. The results of previous studies [74–78] demonstrate a strong and consistent association between the m6A modification and angiogenesis. Patients with type 2 diabetes mellitus have decreased m6A levels and increased expression levels of FTO, METTL3, METTL14, and WTAP mRNA. Of note, m6A levels have been shown to be inversely correlated with the mRNA expression levels of METTL3, METTL14, and FTO. METTL14 and ALKBH5 regulate the m6A modification of TGF-β by mutually controlling and inhibiting the expression level of YTHDF3, an RNA demethylase activity blocker. Because TGF-β is the main causative factor of microvascular lesions in DR, changing its translational efficiency and stability has important effects on DR management. METTL3-mediated hypoxia induces the m6A modification by interacting with YTHDF1, promotes angiogenesis, and regulates the translation of genes responsible for Wnt signaling. WTAP inhibits angiogenesis in endothelial cells. FTO regulates endothelial cell function and ocular angiogenesis in an m6A- and YTHDF2-dependent manner. Its overexpression regulates both angiogenic and fibrotic pathways. Qi *et al*. [79] experimented with streptozotocin-induced mice (DR model) and eventually found that lysine acetyltransferase 1 triggers YTHDF2-mediated Integrin β1 (ITGB1, positively associated with microvessel density and VEGF) mRNA instability to alleviate the progression of DR. Another study [80] showed specific depletion of METTL3 in pericytes suppressed diabetes-induced pericyte dysfunction and vascular complication by constructing pericyte-specific Mettl3 knockout mice. METTL3 overexpression impaired pericyte function by inhibiting the expression of PKC-η, FAT4, and PDGFRA, which was mediated by YTHDF2-dependent mRNA decay. METTL3-YTHDF2-PKC-η/FAT4/PDGFRA signaling axis could be therapeutically targeted for treating microvascular complications.

Retinal microglia, a kind of tissue-resident macrophage, was the major inflammatory cell in the development of diabetic retinopathy [81]. Diabetics rats were constructed and the M1/M2 polarization of retinal microglia was determined using immunofluorescence, flow cytometry, and qRT-PCR. Chen *et al*. [82] discovered that with the increase of glucose concentration, microglia tend to polarize into M1 inflammatory type rather than M2 anti-inflammatory type. Glucose-treated microglia have lower levels of A20 (a potent anti-inflammatory molecule) expression, which have been shown to be negatively correlated with M1 polarization. The Western blot results showed that the expression level of ALKBH5 in microglia after glucose treatment decreased, while METTL3, METTL14 and FTO remained unchanged. These results showed that under high-glucose conditions, downregulation of A20 by ALKBH5-mediated m6A modification leads to enhanced polarization of M1 microglia.

Retinal pigment epithelial (RPE) cells are the main cells of the retina and are widely used as *in vitro* cell models of DR [83]. A detailed examination of the m6A modification in DR by Zha *et al*. [84] found that high glucose levels inhibited ARPE-19 cells (human RPE cell lines) proliferation and promoted apoptosis and pyroptosis in a time-dependent manner. Both METTL3 mRNA and miR25-3p are present at lower levels in the peripheral venous blood samples of patients with diabetes mellitus than in those from normal volunteers. This suggests that high glucose levels may inhibit METTL3 and miR-25-3p expression in RPE cells. As expected, the upregulation of METTL3 and miR-25-3p attenuates the cytotoxic effects of high glucose levels on RPE cells, whereas the knockdown of METTL3 and miR-25-3p has the opposite effect. METTL3 overexpression increases miR-25-3p levels in RPE cells in a microprocessor protein DiGeorge syndrome critical region 8 (DGCR8)-dependent manner, and miR-25-3p ablation abolishes the effect of METTL3 overexpression on high-glucose-treated RPE cells. Moreover, phosphatase and tensin homolog (PTEN) has been identified as a tumor suppressor that inhibits the development of various tumors, and PTEN regulation suppresses the growth of retinal vascular endothelial cells by inactivating the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway [85]. Notably, the PTEN/AKT axis is a downstream target of miR-25-3p, PTEN may be negatively regulated by miR-25-3p, and the overexpression of METTL3 increases phosphorylated AKT levels by targeting the miR-25-3p/PTEN axis [86]. Consistently, PTEN upregulation abolishes the protective effects of METTL3 overexpression

in high-glucose-treated RPE cells. In addition, Gu *et al.* [87] found that miR-192 was weakly expressed in high-glucose-treated ARPE-19 cells. Overexpression of miR-192 abrogated the role of high glucose in RPE cell pyroptosis. What's more, FTO was demonstrated to be a direct target of miR-192. FTO enhanced Nucleotide-binding domain leucine-rich repeat family protein 3 (NLRP3) expression by facilitating demethylation of NLRP3. NLRP3 is vital for cell pyroptosis. Additionally, upregulation of FTO eliminated the effects of miR-192 on RPE cells treated with high glucose. In conclusion, the present results demonstrate that miR-192 represses RPE cell pyroptosis triggered by high glucose via regulation of the FTO/NLRP3 signaling pathway. In another study, Huang *et al.* [88] indicated that circFAT1 bound to YTHDF2 and overexpression of YTHDF2 remarkably increased the expression of LC3B, an autophagy-related molecule, in high glucose-induced ARPE-19 cells. Mechanistically, circFAT1 promoted autophagy and inhibited pyroptosis of RPE cells by binding to YTHDF2.

Overall, the results of these studies outline the critical role of m6A modification in DR. Evidence on angigenesis suggests that regulating FTO, METTL3, METTL14, and WTAP to increase m6A levels may reduce angiogenesis. In addition, the KAT1/YTHDF2/ITGB1 pathway or the METTL3-YTHDF2-PKC-η/FAT4/PDGFRA signal axis can be targeted to alleviate the progression of DR. Evidence on inflammation suggests that regulating ALKBH5 levels reduces m6A modification levels, improves the stability of mRNA, increases A20 expression, and thus relieves retinal inflammation. In addition, to regulate the miR-192/FTO/NLRP3 signal pathway, target the dgcr8-dependent miR-25-3p/PTEN/AKT signal cascade or increase LC3B by overexpressing YTHDF2, may inhibits apoptosis of RPE cells. There are many pathogenesis associated with m6A in DR, and so far we have only found some of them, which may be potential directions for drug development for DR therapies.

2.8 The m6A Modification and Proliferative Vitreoretinopathy

Broadly Proliferative vitreoretinopathy (PVR) can be divided into two groups, one is a long-standing preexisting rhegmatogenous retinal detachment (RRD) and the other is PVRs that occur after initial surgery in patients with RRDs. These events trigger a complex pathogenic mechanism by bringing RPE cells into the vitreous cavity, leading to membrane formation and ultimately to PVR complications [89]. EMT is considered a key pathological mechanism of PVR, and TGF-β is a pivotal growth factor known to induce EMT of RPE cells [90]. In recent years, scientists have found that METTL3 plays different roles in suppressing or promoting EMT in different cancer cells [91].

Recently, Ma *et al.* [92] observed that METTL3 expression decreased in PVR membranes compared with normal RPE membranes in human tissues. They used TGF-β to induce EMT in ARPE-19 cells and found that the expression of m6A and METTL3 was significantly downregulated, while after transfection with METTL3 overexpression plasmid lentiva, they obtained diametrically opposite results. In addition, they detected a higher proportion of cells in the G0/G1 phase when overexpressing MELLT3, suggesting that they inhibited the proliferation of ARPE-19 cells by inducing a G0/G1 block. *In vivo*, intravitreal injection of mettl3-expressing cells in vitreous delayed the development of PVR compared to cells injected into the control group.

In summary, this study suggests that METTL3 is involved in the PVR process, overexpressing METTL3 inhibits the EMT of ARPE-19 cells *in vitro* and inhibits the PVR process *in vivo*. This regulatory mechanism will provide new ideas for the treatment of PVR.

2.9 The m6A Modification and Ocular Melanomas

Ocular melanomas include uveal melanoma (UM) and conjunctival melanoma (CM). It is the most common primary ocular tumor and the second most common type of melanoma in adults. Approximately 95% of ocular melanomas are intraocular and originate from the uvea, whereas the remaining 5% are located in the conjunctiva [93]. Previous studies have shown that epigenetic drivers, such as DNA methylation, histone modification, miRNAs, and long noncoding RNAs are also involved in the tumorigenesis of ocular melanoma [94]. There is a study provided an m6A regulators-based signature for prognostic prediction of UM and confirmed that m6A regulators and related lncRNAs played an important role in tumor microenvironment remodeling [95]. Recently studies have highlighted the critical role played by m6A methylation enzymes in the potential malignant progression and prognosis of UM, and thus, they may be regarded as novel and promising biomarkers [96].

To investigate the functional role of the m6A modification in malignant ocular melanomas, Luo *et al.* [97] examined total m6A levels in ocular melanomas relative to their levels in normal control samples. Human UM cell lines M17, M21, M23 and SP6.5 were isolated from patients with primary choroidal melanoma. Inhibiting METTL3 expression and thereby downregulating m6A methylation inhibits proliferation, migration, and invasion of UM cells. c-Met is a cell surface tyrosine kinase and a well-defined driver of oncogenesis in various types of cancer. Downregulated m6A (Cyc treatment or knockdown of METTL3) led to the downregulation of c-Met protein levels but had no significant effect on c-Met mRNA levels in UM cells. This suggests that m6A modification regulates c-Met expression by influencing translation, rather than transcription. What's more, they discovered a key downstream molecule in the downregulated signaling pathways is Akt, which is vital for cell survival and migration. In short, it demonstrated

that MELLT13 exerts a role in promoting UM progression through the m6A/c-Met/Akt axis.

In another study, Hao *et al*. [98] investigate the association between ALKBH5 expression and UM, they measured ALKBH5 expression in two human UM cell lines (MuM-2B and C918). The expression of ALKBH5 was significantly increased in UM cells. Futhermore, they demonstrated that inhibition of ALKBH5 suppresses tumor growth *in vivo* and EP300-induced H3K27ac activation promotes ALKBH5 expression. They also looked at the relationship between ALKBH5 and EMT. The Western blot test results showed that the downregulated catalytic activity of ALKBH5 increased the epithelial cell phenotype marker E-cadherin and decreased mesenchymal phenotype markers (N-cadherin and Vimentin) compared with controls. Furthermore, ALKBH5 can promote EMT by increasing the expression and mRNA stability of FOXM1 (a transcription factor that promotes EMT).

Besides, Wang *et al*. [99] demonstrated that RBM15B was the sole independent prognostic factor for UM overall survival, via analysis of the GEPIA2 database and multivariate cox regression. They confirmed that RBM15B can inhibit UM growth and progression. Gene Ontology enrichment analysis implying that RBM15B expression was primarily correlated with immune-related gene terms. Then they used ENCORI and OncomiR databases to filter out potential LncRNA and miRNA and construct LINC00665/hsa-let-7b-5p/RBM15B axis and LINC00638/hsa-miR-103a-3p/RBM15B axis, which are potential prognostic biomarkers in UM.

Integral membrane glycoprotein beta-secretase 2 (BACE2), an enzyme that cleaves amyloid precursor proteins into amyloid peptides, has been reported to play important roles in vertebrate pigmentation and the development of metastatic melanomas [100]. He *et al*. [101] analyzed RNA sequence data from human ocular melanoma cells and normal control cells and found that BACE2 was significantly upregulated in both UM and CM cell lines. Subsequently, BACE2 expression was examined using a tissue chip, and it was found to be significantly upregulated in ocular melanoma samples. In addition, He *et al*. [101] designed two small hairpin RNAs (shBACE2-1 and shBACE2-2, with an EGPF tag) for BACE2-silenced MUM2B melanoma cells into nude mice and found that BACE2 inhibition significantly impaired tumor progression *in vitro* and *in vivo*. Significant inhibition of migration and cell growth were observed after BACE2 silencing in ocular melanoma cells, and BACE2-silenced ocular melanoma cells formed fewer and smaller colonies than control cells. Notably, transmembrane protein 38 B (TMEM38B), whose expression is highly dependent on BACE2, regulates calcium release from the endoplasmic reticulum. Inhibition of the BACE2/TMEM38B axis triggers the inhibition of intracellular calcium release and tumor progression. To determine the cause of BACE2 upregulation in ocular melanoma, meRIP-seq and quantitative PCR analyses were performed. Significantly increased m6A methylation of BACE2 and a significant positive association between METTL3 and BACE2 expression levels were observed. After METTL3 silencing, significant decreases in BACE2 RNA methylation and BACE2 expression at both the RNA and protein levels were observed.

In summary, METTL3, ALKBH5, and global m6A levels are highly correlated with the progression and prognosis of ocular melanoma. MELLT13 exerts a role in promoting UM progression through the m6A/c-Met/Akt axis. ALKBH5, a potential target of UM molecular therapy, which is positively regulated by epigenetic modifications of H3K27 acetylation, promotes tumor progression by inducing tumor EMT and increasing FOXM1 (Forkhead box protein M1) expression via m6A demethylation. RBM15B was the sole independent prognostic factor for UM overall survival. LINC00665/hsa-let-7b-5p/RBM15B axis and LINC00638/hsa-miR-103a-3p/RBM15B axis are potential prognostic biomarkers in UM. In addition, METTL3/m6A/BACE2/TMEM38B axis may be a potential therapeutic target for ocular melanoma. It should be noted that these studies have some limitations of having no validation experiments on human samples, and the lack of more clinical data and experiments.

2.10 The m6A Modification and Retinoblastoma

Retinoblastoma (RB) represents 3% of all childhood cancers, and is the most common intraocular malignancy of childhood. It may have devastating consequences, including blindness and even death [102]. Due to the poor prognosis of RB, it is crucial to develop effective diagnostic and therapeutic strategies. Previous studies [103] have shown that METTL3 is closely associated with the occurrence and development of cancer. However, whether METTL3 is associated with RB remains unclear.

To investigate the role of METTL3 in RB regulation, Zhang *et al*. [104] first analyzed METTL3 expression in RB patients and found that it was expressed in RB tumor samples. Since RB originates in the developing retina, the mRNA and protein levels of METTL3 in normal ARPE-19 cells and the RB cell lines, Y79 and WERI-Rb-1, were further compared by quantitative PCR and western blotting, respectively. The results showed that METTL3 mRNA and protein levels were higher in the two RB cell lines than in normal ARPE-19 cells. Thus, METTL3 may regulate the progression of RB. In further experiments in RB cell lines with METTL3 knocked down, the apoptosis rate increased by more than 50%, and migration and invasion were significantly inhibited. In contrast, the upregulation of METTL3 by the transfection of lentiviral constructs increased the proliferation, decreased the apoptosis rate by more than 50%, and significantly enhanced the migration and invasion of RB cells. This suggests that the upregulation of METTL3 promotes RB progression, whereas the downregulation of

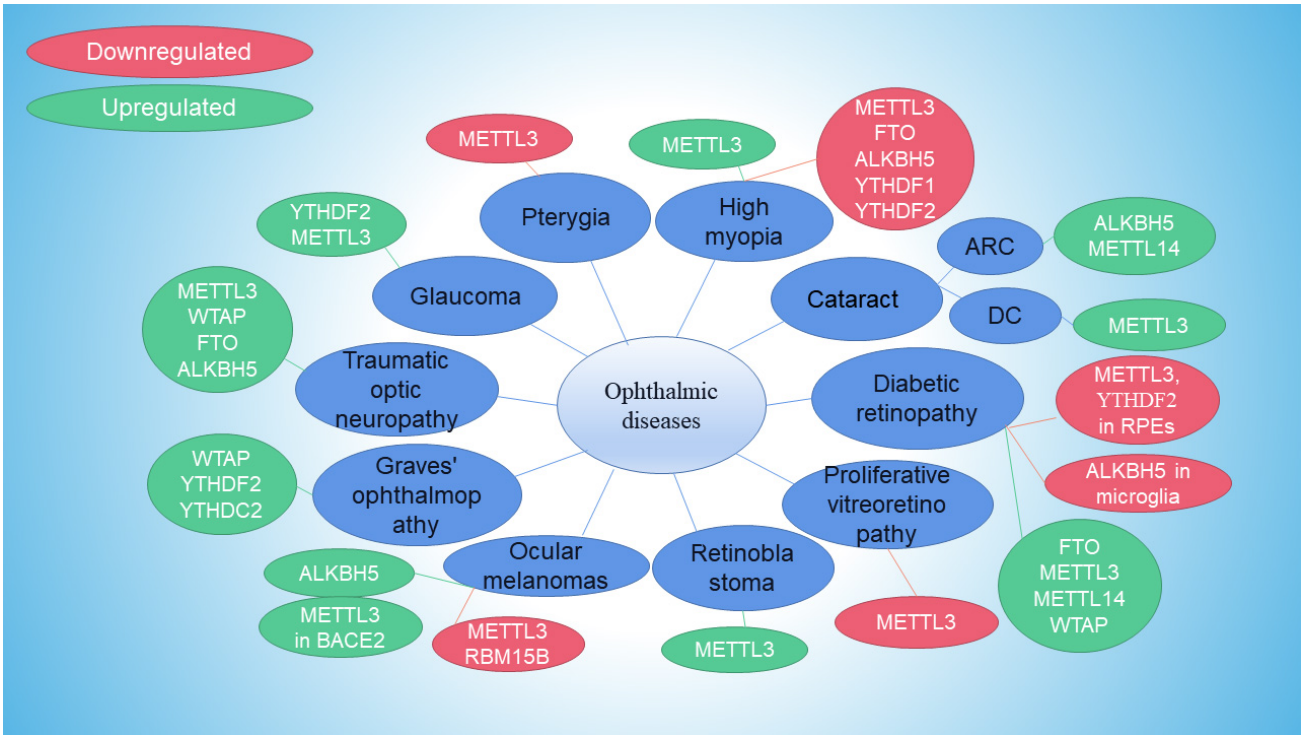

Fig. 2. Expression levels of m6A enzyme in ophthalmic diseases. The blue color is 10 ophthalmic diseases; the green color is the m6A enzyme that expresses the level of upregulation in the corresponding ophthalmic disease; the red color is the m6A enzyme that expresses the level down-regulation in the corresponding ophthalmic disease.

METTL3 has the opposite effect. Furthermore, PI3K-p85, AKT, mammalian target of rapamycin (mTOR), and 70-kDa ribosomal protein S6 kinase (P70S6K) phosphorylation levels decreased in METTL3-downregulated cells, whereas PI3K-p85, AKT, mTOR, P70S6K, 4EBP1 mRNA levels increased. These findings suggest that METTL3 regulates cell proliferation, migration, and invasion via the PI3K/AKT/mTOR signaling pathway. Finally, subcutaneous xenograft mouse models have confirmed the effects of METTL3 *in vivo*.

Taken together, these findings show that METTL3 knockdown reduces RB cell proliferation, migration, invasion, and tumorigenesis *in vitro* and *in vivo*, whereas METTL3 overexpression promotes the progression of RB. The evidence reviewed here suggests that METTL3 regulates cell proliferation, migration, and invasion via the PI3K/AKT/mTOR signaling pathway. This suggests that targeting PI3K/AKT/mTOR signaling pathway may be a promising therapeutic strategy for the treatment of RB.

3. m6A-Modification-Related Potential Therapeutic Strategies in Ophthalmic Diseases

As an important epigenetic regulatory mechanism for RNA, m6A modification is implicated both in the normal development of ocular tissues and in the appearance and development of various ophthalmic diseases. Furthermore, m6A methylation is reversible, and medicines that induce demethylation can reshape gene expression. If a disease is associated with hypermethylation, therapeutic effects may be achieved after intervention with drugs that induce demethylation. Targeting related mechanisms is also an effective treatment strategy. However, the regulatory role of m6A in ophthalmic diseases is a new field that has not previously been reviewed. Thus, a summary of m6A modification-related ophthalmic diseases and potential therapeutic strategies may make an important contribution to the field of ophthalmic disease treatment. This article reviews recent advances in ophthalmic disease research related to m6A modification and suggests possible therapeutic strategies (Fig. 2; Table 2, Ref. [41,49,54,57,58,63–66,69,72,74–80,82,84–88,92,97–99,101,104]).

However, much uncertainty still exists regarding the regulatory mechanism of m6A modifications in ophthalmic diseases. Systemic administration of drugs that induce demethylation causes genome-wide hypomethylation, which results in side effects. For example, m6A methylation is a double-edged sword in ophthalmic diseases caused by diabetes. In DR, low METTL3 expression levels promote apoptosis in RPE cells, whereas in DC, high METTL3 expression levels inhibit human lens epithelial cells proliferation and promote their apoptosis [105].

Traditional medicine-based natural products are reliable sources for the discovery of new therapeutic agents targeting m6A modification. Deng *et al*. [106] Summarize

Table 2. The roles of m6A modification in ophthalmic diseases.

Ophthalmic diseases		m6A enzymes	Expression	Potential therapy strategies	The biological effects	References
Pterygium		METTL3	Downregulated	Upregulate METTL3 to increase the m6A level may change relative genes	Reduce the occurrence of pterygia	[41]
High myopia		METTL14 METTL3 FTO ALKBH5 YTHDF1 YTHDF2	Upregulated Downregulated	Regulate related m6A enzymes to decrease the m6A level	Contribute to the prevention and treatment of high myopia complications	[49]
Graves' ophthalmopathy		WTAP YTHDF2 YTHDC2	Upregulated	Target correlative m6A enzymes to reduce the m6A level	Delay the development of Graves' ophthalmopathy	[54]
Glaucoma		YTHDF2	Upregulated	YTHDF2 cKO	Protects retinal ganglion cells from dendritic degeneration	[57]
		METTL3	Upregulated	Target the METTL3/Smad3/TGF-1 signaling pathway	Attenuates scar formation in glaucoma	[58]
Traumatic optic neuropathy		METTL3 WTAP FTO ALKBH5	Upregulated	Regulate MAPK and NF-κB signaling pathways	Postpone the progression of optic nerve damage	[63–66]
Cataract	ARC	ALKBH5 METTL14	Upregulated	Downregulate ALKBH5 to increase the expression of m6A-circRNAs	Reduce the apoptosis of lens epithelium cells	[69]
	DC	METTL3	Upregulated	Downregulate METTL3 to reduce the stability of ICAM-1	Inhibits the apoptosis of lens epithelium cells	[72]
Diabetic retinopathy		FTO METTL3 METTL14 WTAP	Upregulated	Regulate related m6A enzymes to increase the m6A level; target KAT1/YTHDF2/ITGB1 pathway or METTL3-YTHDF2-PKC-η/FAT4/PDGFRA signaling axis	Reduce angiogenesis	[74–80]
		ALKBH5 in microglia	Downregulated	Regulate the ALKBH5 level to reduce the m6A level, enhance the mRNA stability and increase the expression of A20	Alleviate retinal inflammation	[82]
		METTL3 and YTHDF2 in RPEs	Downregulated	Target DGCR8-dependent miR-25-3p/PTEN/Akt signaling cascade; upregulate YTHDF2 to increase LC3B	Promoted autophagy and inhibited pyroptosis of RPE cells	[84–88]
Proliferative vitreoretinopathy		METTL3	Downregulated	Upregulate METTL3 to inhibited proliferation	Delayed the development of PVR	[92]
Ocular melanomas		METTL3	Downregulated	Upregulate METTL3 and downregulate ALKBH5 to increase the m6A level	Inhibit the migration ability and cell growth rate of melanoma cells	[97]
		ALKBH5	Upregulated	Downregulate ALKBH5 to decrease the m6A level	Inhibit tumor EMT and decrease FOXM1 expression	[98]
		RBM15B	Downregulated	Target LINC00665/hsa-let-7b-5p/RBM15B and LINC00638/hsa-miR-103a-3p/RBM15B axis	Inhibit UM growth and progression	[99]
		METTL3	Upregulated in BACE2	Target METTL3/m6A/BACE2/TMEM38b signaling axis	Trigger the depletion of intracellular calcium release	[101]
Retinoblastoma		METTL3	Upregulated	Target the METTL3/PI3K/AKT/mTOR signaling axis	Reduces RB cell proliferation, migration, invasion, and tumorigenesis	[104]

the current common traditional medicine-based natural products. Curcumin, a natural phenolic compound, can reduce the expression of ALKHB5. Combining resveratrol with curcumin effectively decreases m6A and enhanced YTHDF2 [107]. Quercetin has a synergistic effect with cisplatin on inhibiting the expression of METTL3 [108]. The baicalin hydrate upregulates m6A RNA methylation, as evidenced by increased METTL3 and METTL14 and decreased FTO and ALKBH5 [109]. Epigallocatechin gallate is a tea flavonoid which may be associated with the regulation of cyclin A2 and CDK2 in an m6A-dependent manner mediated by inhibiting the expression of FTO and increasing expression of YTHDF2 [110]. Betaine suppressed the expression of the m6A methylases METTL3 and METTL14 but facilitated the expression of the demethylases FTO and ALKBH5 [111]. Clausine E dose-dependently inhibited the demethylation activity of FTO. Rhein, an anthraquinone rich in Rheum rhabarbarum, was identified as the first cell-active reversible and competitive inhibitor of FTO [112]. Saikosaponin is a classical triterpenoid that is extracted from Radix Bupleuri, which can inhibit FTO to rescue m6A hypomethylation [113]. Furthermore, it would be more efficient to develop novel and effective therapeutic agents that inhibit m6A modification-mediated tumor progression by combining traditional medicine-based natural products databases with artificial intelligence-based drug discovery approaches. It will reduce the cost and shorten the time of drug development related to m6A modification [114].

Recently, FTO and METTL3 have attracted considerable interest as potential targets of cancer therapy. Small-molecule FTO inhibitors (for example, mechlorfenac sodium) have been shown to prevent tumor progression in acute myeloid leukemia (AML) and glioblastoma *in vivo*. A comprehensive analysis and discussion of the subject has been presented by Su *et al*. [115], who showed that FTO regulates MYC and CCAAT enhancer-binding protein alpha expression and that the inhibition of FTO by (R)-2-hydroxyglutaric acid decreases the proliferation and viability of leukemia cells *in vitro* and *in vivo*. Cui *et al*. [116] confirmed that FTO inhibition inhibits tumor progression and significantly prolongs the life span of mice transplanted with glioblastoma stem cells. However, currently available FTO inhibitors are not suitable for clinical use because of their poor target selectivity or poor pharmacokinetic properties. A recent study [117] reported that the FTO inhibitor, FTO-04, which prevents neurosphere formation in glioblastoma stem cells without inhibiting the growth of healthy neurostem cell-derived neurospheres, is a potentially new therapeutic agent for glioblastoma. In addition to FTO inhibitors, METTL3 inhibitors may serve as a potential therapeutic agents for AML [118]. It has been demonstrated that the treatment of tumors with STM2457, a highly efficient and selective first-class METTL3 catalytic inhibitor, leads to reduced AML growth and differentiation, increased apoptosis, impaired implantation, and prolonged survival in various mouse models of AML. However, the development of more m6A targeted therapy drugs is still focused on cancer, and there are no relevant reports in ophthalmic diseases.

Although previous studies have reported the significant role of FTO and METTL3 in ophthalmic diseases, there has not been a systematic investigation of the potential therapeutic effects of FTO and METTL3 inhibitors in ophthalmic diseases. In addition, there are currently no studies on how new drugs targeting m6A regulators can be implanted into eyedrop, ophthalmic ointments or intraocular injections. Furthermore, it may be a good idea to combine it with nanobiotherapy targeted therapy, which can improve accuracy, control drug release rates, increase dosing time, and reduce drug side effects. Previous research [119] has shown that the near infrared light triggers drug delivery system based on black phosphorus hydrogel and that it can achieve precise treatment of cancer. The rate of drug release can be controlled accurately by adjusting a variety of parameters. After treatment, the black phosphorus hydrogel material can be automatically degraded into non-toxic products. Based on these, it is a good idea to build a nano-drug controlled release system integrated with drugs targeting m6A regulators, so as to establish a physical model for the intelligent and precise regulation of drug release rate by the black phosphorus hydrogel drug delivery system.

In this article, the specific mechanism of m6A in various ocular diseases is discussed, which can help people later develop new medicines for ophthalmic diseases targeting m6A regulators. And it's a great idea to combine traditional medicine-based natural products databases with artificial intelligence-based drug discovery approaches to develop new targeted drugs. In addition, cellular or animal experiments on the therapeutic effects of new medicines targeting m6A regulators in specific ophthalmic diseases should also be performed. Furthermore, making new drugs targeting m6A regulators into eyedrop, eye ointment, intraocular injections or building a nano-drug controlled release system integrated with drugs targeting m6A regulators, are also good research directions.

4. Conclusions

This study covers almost all m6A-related ophthalmic diseases and suggests potential therapeutic strategies for each disease. However, much uncertainty still exists regarding the regulatory mechanism of m6A modifications in ophthalmic diseases. Systemic administration of drugs that induce demethylation leads to whole-genome hypomethylation, resulting in side effects. Thus, the next research objective could be to develop new medicines targeting m6A modulators for specific ocular diseases and to conduct cellular and animal experiments to test the therapeutic effects. Furthermore, making new drugs targeting m6A regulators into eyedrop, eye ointment, intraocular injections or building a nano-drug controlled release system integrated with

drugs targeting m6A regulators, are also good research directions. This review laid the groundwork for future studies.

Author Contributions

These should be presented as follows: XC wrote the manuscript. YX, QL and XL provided help and advice on paper revisions. YG was responsible for ensuring that the descriptions are accurate and agreed by all authors. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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