Review article

N6-methyladenosine methylation in ophthalmic diseases: From mechanisms to potential applications

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ABSTRACT

N6-methyladenosine (m^6A) modification, as the most common modification method in eukaryotes, is widely involved in numerous physiological and pathological processes, such as embryonic development, malignancy, immune regulation, and premature aging. Under pathological conditions of ocular diseases, changes in m^6A modification and its metabolism can be detected in aqueous and vitreous humor. At the same time, an increasing number of studies showed that m^6A modification is involved in the normal development of eye structures and the occurrence and progress of many ophthalmic diseases, especially ocular neovascular diseases, such as diabetic retinopathy, age-related macular degeneration, and melanoma. In this review, we summarized the latest progress regarding m^6A modification in ophthalmic diseases, changes in m^6A modification-related enzymes in various pathological states and their upstream and downstream regulatory networks, provided new prospects for m^6A modification in ophthalmic diseases and new ideas for clinical diagnosis and treatment.

1. Introduction

Until now, over 160 types of RNA modifications have been discovered [1], including N6-methyladenosine (m^6A), N1-methyladenosine (m^1A), 5-methylcytosine (m^5C), 5-hydroxymethylcytosine (hm5C), pseudouridine (Ψ), inosine (I), uridine (U), ribose methylation (2′-O-Me) [2], N7-methylguanosine (m^7G), and N4-acetylcytidine (ac4C) [3], which have a close connection with multiple physiological and pathological processes, such as embryonic development [4], cancer development and progression [5], learning and memory [6], angiogenesis, and immunity [7]. Meanwhile, some modifications, such as N2-methylguanosine (m^2G) [8] and 5-methyluridine (m^5U) [9], can be detected in tRNAs and rRNAs.

Among these different modification manners, m^6A is the most abundant. Almost 0.2%–0.6% of total adenosines are occupied by m^6A modification in mammalian RNA [2], accounting for over 80% of all RNA methylation modifications [10]. On average, there are 2–3 m^6A-modified sites at a transcript [11]. m^6A regulation is dynamic and reversible, functioning by methyltransferase, demethylase,

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and methylation recognition reader. Methyltransferase complex (MTC) is necessary for the addition of methyl groups, including METTL3, METTL14, WTAP, RBM15, VIRMA, HAKAI, and ZC3H13 [12]. Demethylases mainly include ALKBH5 and FTO. The YTH and IGF2BP families are the readers of m^{6}A modification, which recognize methylated substrate molecules through a consensus "DRACH" motif (D = A/G/U, R = A/G, H = A/C/U), 3' and 5' untranslated regions are the main modification positions [13]. m^{6}A regulation contributes to mRNA splicing, export, stability, translation, and degradation, as well as the processing of miRNA precursors [14].

The accumulated evidence is sufficient to illustrate the point that m^{6}A modification is closely related to the onset and development of ocular diseases. Actively exploring the pathological mechanisms and regulatory networks of m^{6}A modification in ophthalmic diseases will provide new ideas and guidance for clinical diagnosis and treatment. In this review, we summarized the regulatory role of m^{6}A modification in various ophthalmic diseases (Table 1) and provided directions for future research.

2. m^{6}A modification in ophthalmic diseases

2.1. Retinal and choroidal diseases

2.1.1. Retinal development and related diseases

As the core methylase complex in m^{6}A modification, METTL3, METTL14, and WTAP are highly expressed in the development of zebrafish retina, and knocking down this complex in zebrafish embryo leads to microphthalmia, delayed differentiation of retinal precursor cells, and increased apoptosis of retinal cells [15]. The specific knockdown of METTL3 in retinal precursor cells inhibits the degradation of transcripts, blocks the transformation of retinal precursor cells to Müller cells, and induces distortion of Müller cells, retinal structural disorder, and visual function damage in mice after birth [16]. The specific knockdown of METTL14 in photoreceptor cells can eventually lead to the distortion and degeneration of rod and cone cells, down-regulated dark adaptation, and decreased expression of light signal conduction-related genes by inhibiting the formation of photoreceptor cilia and the transport defect of OS

Table 1
Alterations of m^{6}A enzymes and signaling pathways in ophthalmic diseases.

Disease	Changes in m^{6}A enzymes	Molecular targets/signal pathway	Reference
GO	WTAP, ALKBH5, ELF3, YTHDF2, YTHDC2 ↑	–	[15]
pSS	ALKBH5, RBMX, RBM15B, YTHDF1 ↓	–	[16,17]
Corneal angiogenesis	METTL3 ↑	YTHDF1-LRP6/DVL1-Wnt axis	[18]
	METTL3 ↓	AHNAK/DDIT4	[19]
	FTO ↓	FAK-YTHDF2	[20]
Fungal keratitis	METTL3, METTL14↑	PI3K-AKT-IL-1β/IL-6/TNF-α axis; NF-κB/TRAF6 axis	[21,22]
Pterygium	METTL3, FTO, YTHDF1 ↓	DSP, MXRA5, ARHGAP35, TMEM43, OLFML2A	[23]
Glaucoma	METTL3 ↑	Smad3	[24]
	YTHDF2 ↑	–	[25]
Cataract with high myopia	METTL14 ↑ , METTL3, FTO, ALKBH5, YHTDF1, YTHDF2 ↓	–	[26]
ARC	ALKBH5 ↑	–	[27]
Diabetic cataract	METTL3 ↑	ICAM-1	[28]
Ocular melanoma	METTL3 ↑	BACE2-TMEM38B; c-Met/AKT pathway	[29,30]
	METTL14 ↑	RUNX2-Wnt/β-catenin axis	[31]
	ALKBH5 ↑	EP300-H3K27ac-FOXM1	[32]
	YTHDF2 ↑	H3K18la-YTHDF2-PER1/TP53	[33]
	YTHDF3 ↑	CTNNB1	[34]
Uveitis	METTL3 ↓	ASH1L-YTHDC2-IL17/IL24R	[35]
	FTO ↓	ATF4-STAT3	[36]
	YTHDC1 ↑	SIRT1-STAT3 axis	[37]
Retinal dysplasia	METTL3/METTL14/WTAP ↓	Dysfunction of retinal precursor cells and photoreceptor cells	[38–40]
OIR	METTL3 ↑	LRP6/DVL1-YTHDF1	[18]
DR	METTL3 ↑	PKC-η/FAT4/PDGFRA-YTHDF2	[41]
	METTL3 ↓	CYP2J2-ANXA1; SNHG7-KHSRP-MKL1; Wnt/β-catenin axis	[42,43,44]
	ALKBH5 ↓	A20	[45]
	YTHDF2 ↓	PARP1; circFAT1; KAT1-ITGB1-FAK-PI3K-AKT axis;	[40,41,43]
AMD	METTL3 ↑	NR2F1	[50]
	FTO ↓	PKA-CREB/brain-derived neurotrophic factors	[51]
	YTHDC1 ↓	miR-145-5p/CDKN1A	[53]
TON	METTL3, WTAP, FTO, ALKBH5 ↑	–	[57]
RP	METTL14 ↓	YTHDF2-MAP2-NEUROD1	[45]
Retinoblastoma	METTL3 ↑	PI3K-AKT-mTOR-P70S6K-4EBP1 axis;	[75]

Abbreviations: AMD, age-related macular degeneration; ARC, age-related cataract; DR, diabetic retinopathy; GO, Graves' ophthalmopathy; OIR, oxygen-induced retinopathy; pSS, primary Sjögren's syndrome; RP, retinitis pigmentosa; TON, traumatic optic neuropathy.

protein [17].

2.1.2. Retinal neovascular diseases

Retinal neovascular diseases are still the main causes of vision loss and blindness around the world, including diabetic retinopathy (DR), retinopathy of prematurity (ROP), and retinal vein occlusion [18–20]. Therapy with anti-vascular endothelial growth factor (VEGF) agents is the priority recommendation, especially for patients with advanced vascular development [21]. However, it is not 100% effective and might cause subsequent side effects, such as endophthalmitis and retinal detachment [22,23]. Hence, the exploration of brand-new pathological mechanisms and therapeutic targets has great research significance. Hypoxia and ischemia are currently recognized as the launch steps of retinal neovascularization, stimulating the secretion of proinflammatory factors and angiogenesis-related factors, such as interleukin–1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α) and VEGF [24]. Increasing evidence has confirmed that m^{6}A modification regulators play important roles in the development of retinal angiogenesis (Fig. 1). For example, as a classical model for retinal angiogenesis, a mouse model of oxygen-induced retinopathy (OIR) shows significant differences of m^{6}A modification in mRNA, circRNA, and lncRNA compared to the control group [25,26].

Microvascular dysfunction is one of the common features of retinal neovascular diseases, among which defects in the blood-retinal barrier can cause tissue edema, damage, and vision loss [27]. The knockdown of endothelial-specific METTL3 significantly slows down cell viability, proliferation, migration, and tube formation ability. Importantly, mice with the low expression of METTL3 in endothelial cells have smaller avascular and neovascular areas of the retinas than control groups in OIR mice [28]. Pericytes are crucial to the maturation of blood vessels and the maintenance of the integrity of the blood-retinal barrier [29]. Research uncovers that METTL3 could target PKC- η, FAT4, and PDGFRA, suppress pericyte activity, increase apoptosis of cells and leakage of macromolecules after YTHDF2 recognizes m^{6}A modified targets, thus promoting vascular leakage, microaneurysm development and the formation of cell-free capillaries [30]. ANXA1 is essential to maintain the integrity of the blood-brain barrier, the overexpression of CYP2J2 in endothelial cells promotes METTL3 mediated m^{6}A modification of ANXA1, maintains the stability and permeability of retinal blood vessels, and alleviates the blood-retinal barrier function caused by retinal ischemia-reperfusion injury [31]. However, METTL3 is down-regulated in some studies. METTL3 can regulate endothelial mesenchymal transition and enhance cell invasion and migration by mediating the SNHG7/KHSRP/MKL1 signal axis [32], which were evidenced to be potential targets of malignant tumors [33–35]. Low expression of METTL3 and low m^{6}A level modification can mediate the Wnt/β-catenin signaling pathway, promote cell proliferation and migration and epithelial-mesenchymal transition, and promote the formation of the retinal proliferative vascular fibrous membrane [36].

M1 polarization level of microglia in DR rats remarkably increases, and low expression of ALKBH5 leads to an increased level of overall m^{6}A modification in microglia and accelerates the degradation rate of the potent anti-inflammation molecule-A20 [37], and regulating M1 polarization of microglia to maintain the chronic inflammatory response in the eye [38].

According to previous reports, YTHDF2 is primarily responsible for facilitating the decay of targets [39]. YTHDF2 might be involved in the progression of DR by accelerating cell autophagy and inhibiting apoptosis by binding to circFAT1, or reducing the mRNA stability of ITGB1 by mediating the FAK/PI3K/AKT signaling pathway, eventually leading to aberrant retinal inflammatory response, neovascularization, and vascular leakage [40,41]. As a key component of ADP-ribosylation [42], the upregulated PARP1 in DR is uncovered to be a target of YTHDF2, and contributes to promoting inflammation, extracellular matrix aggregation, fibrosis, and angiogenesis [43].

2.1.3. Retinitis pigmentosa (RP)

Mutation in RPGR^{orf15} leads to the X-linked RP, *Appelbaum T* et al. design and identify a new circRNA for the ORF15 exon region
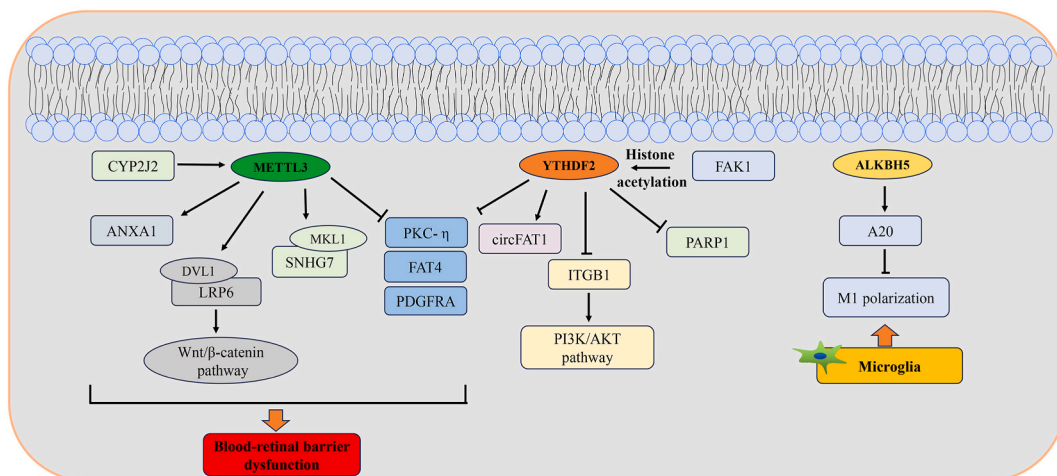

Fig. 1. The regulatory roles of m^{6}A modification in retinal neovascularization.

and find the sequence has a common recognition frame of m6A modification [44]. Simultaneously, the expression of METTL14 is significantly reduced in patients with RP. YTHDF2 could identify METTL14 m6A-modified MAP2 and NEUROD1, regulate retinal pigment epithelial (RPE) cells and reduce phagocytosis, increase apoptosis, block cell cycle and down-regulate cellular tight junction [45].

2.1.4. Age-related macular degeneration (AMD)

As a blinding disease, AMD is closely associated with aging and is the main cause of vision loss among elderly people [46]. It can be divided into dry and wet AMD, the former is characterized as geographic atrophy, and the latter is featured as choroid neo-vascularization [47]. The pathogenesis of AMD is still not fully clarified, hypoxia and insufficient blood supply of choroid, progressive destruction of RPE cells, and abnormal inflammation and immune response are the major reasons [48,49].

The study reports that METTL3 can promote RPE cell proliferation, strengthen cellular tight junction, and decrease the release of inflammatory factors, thus providing new ideas for the research on AMD [50]. The reduction of RPE cytochrome, structural disorder, and progress of AMD are aggravated by the inhibition of FTO, which can regulate the demethylation of PKA and mediate the expression of CREB and brain-derived neurotrophic factor [51]. As a reader of m6A modification, YTHDC1 participates in the biological processes of RNA exporting and splicing [52]. Research reports that YTHDC1 is decreased in patients with AMD and dysfunctional RPE cells. Additionally, depolarization of RPE cells (decreased tight junction between cells and phagocytosis, increased disordered lipid metabolism) could be induced by YTHDC1 and their downstream molecules miR-145-5p/CDKN1A; This will lead to retinal instability (photoreceptor dysfunction, retinal thinning, and activation of microglia) and promote the occurrence of AMD [53].

2.1.5. Uveitis

In autoimmune uveitis, the expressions of various m6A modification enzymes are reduced (METTL3, METTL14, FTO, and ALKBH5), and low expression of FTO leads to an increase in m6A levels, promotes cell proliferation and inflammatory factor release and reduces cellular tight junctions by mediating the ATF4/STAT3 pathway [54]. Studies further confirmed that the low expression of METTL3 regulates the ASH1L/YTHDC2/IL17/IL24R signal pathway, activates autoreactive IL17 cells, and exacerbates the inflammatory response [55]. At the same time, YTHDC1 expression in retinal microglia in uveitis is significantly down-regulated, which influences the SIRT1/STAT3 signaling pathway to promote migration and M1 polarization of microglia [56].

2.1.6. Optic neuropathy

Aberrant m6A modification exists in the retina of a rat model of traumatic optic neuropathy (TON) shown by methylated RNA immunoprecipitation sequencing (MeRIP-seq) [57]. In this study, METTL3, WTAP, FTO, and ALKBH5 are significantly up-regulated after TON. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis shows the involvement of MAPK, NF-κB and TNF pathways, which are closely related to inflammation [57].

2.2. Ocular tumors

2.2.1. Melanoma

Ocular melanoma is the most common highly malignant ocular tumor in adults and the second most common melanoma in the whole body, and has a poor prognosis [58]. The incidence rate of ocular melanoma is 85.0%, 4.8%, and 10.2% in uveal, conjunctival, and other sites, respectively [59]. Regarding the onset and progress of ocular melanoma, the most common events are mutational activation of G protein subunit alpha Q (GNAQ) or G protein subunit alpha 11 (GNA11) [60], the loss of chromosome 3, the loss of the BRACA1-associated protein 1 (BAP1) [61], impaired EIF1AX and SF3B1 [62], and immune system dysfunction [63]. Although there
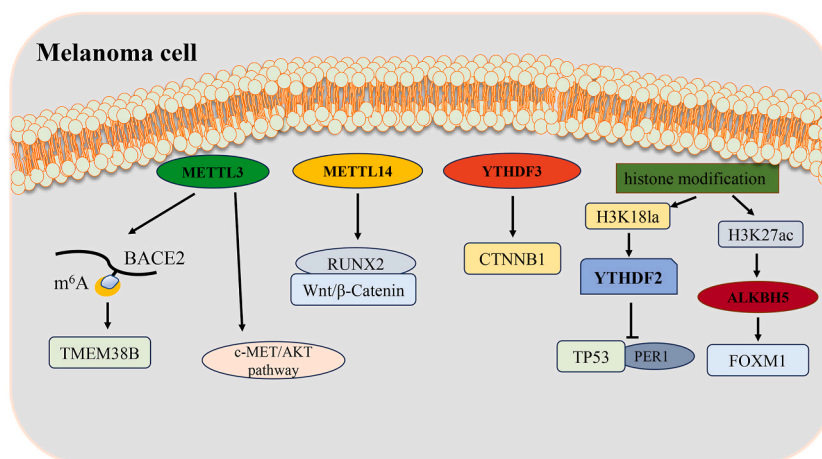

Fig. 2. The regulatory roles of m6A modification in ocular melanoma.

are many treatments for ocular melanoma, such as radiotherapy, enucleation [61], and combined immune checkpoint blockade [64], its high malignancy, high metastasis rate, and related complications make it imperative to find the potential molecular mechanism. The involvement and mechanisms of m^{6}A modification in ocular melanoma have been summarized in Fig. 2.

Increased evidence has shown that m^{6}A modification plays an important role in ocular melanoma. A study divides uveal melanoma (UM) into C1 and C2 groups based on the clinical characteristics of ocular melanoma (C1 group has a greater tendency toward adverse outcomes), finds differentially expressed m^{6}A modification-related enzymes (METTL3, METTL14, WTAP, ALKBH5, YTHDF1, YTHDC1, YTHDC2, ZC3H13, KIAA1429, and RBM15), and shows that SE3BI could significantly change their expressions [65]. In another study, RBM15B, IGF2BP1, IGF2BP2, YTHDF1, and YTHDF3 are related to the prognosis of UM, and RBM15B is the only independent prognostic factor [66].

High m^{6}A modification of BACE2 by METTL3 in UM and conjunctival melanoma (CM) tissues target TMEM38B, which could result in increasing intracellular calcium release and epithelial-mesenchymal transformation [67]; Furthermore, METTL3 mediates c-Met/AKT signaling pathway and significantly upregulates overall m^{6}A level of UM [68]. The overexpression of METTL14 in choroidal melanoma mediates m^{6}A modification of RUNX2, activates the Wnt/β-catenin pathway, and promotes the growth and metastasis of tumor cells in vivo and in vitro [69]. YTHDF3 maintains the characteristics of melanoma stem cells of UM and CM by targeting CTNNB1 and enhancing its translation [70].

There is a monumental scientific breakthrough showing that histone modification is bridged with m^{6}A modification in ocular melanoma. High expression of ALKBH5 in UM could be increased by EP300-induced H3K27 acetylation (H3K27ac), and influence downstream target FOXM1, increase cell migration and cell viability, promote the percentage of cells in the S stage, and induce the epithelial-to-mesenchymal transformation [71]. *Yu* et al. discover that the overall lactylation level significantly increases in UM and CM, especially for the histone H3K18 lactylation (H3K18la), which is positively correlated with the malignant degree of tumor [72]. Meanwhile, global histone lactylation level could positively change the expression of YTHDF2, and YTHDF2 decreases mRNA stability of two tumor suppressors, PER1 and TP53, which make YTHDF2 have a negative association with ocular melanoma prognosis [72].

2.2.2. Retinoblastoma

Retinoblastoma is the most common eye malignancy in children, and the prognosis remains poor in low and middle countries [73]. The development of retinoblastoma is caused by biallelic mutation of retinoblastoma gene (RB1) in a susceptible retinal cell and retinoblastoma protein (pRB) can not perform its functions [74]. Research reported that abnormally elevated expression of METTL3 has the ability to control survival, migration, and invasion of retinoblastoma cells and accelerate the growth and progression of retinoblastoma by targeting PI3K/AKT/mTOR/P70S6K/4EBP1 signaling pathway [75].

2.3. Corneal diseases

2.3.1. Corneal angiogenesis

Transparency and avascularity are the two main characteristics of the cornea, and they are the premise conditions of good visual acuity [76]. Infection, inflammation, hypoxia, corneal degeneration, and immune-mediated rejection are the common reasons for corneal neovascularization [77]. Additionally, angiogenesis is mainly controlled through anti-inflammatory treatment, physical vessel ablation, corneal transplantation, and anti-VEGF agents [77]. One study evidences that high expression of METTL3 and m^{6}A modification level target LRP6 and DVL1, and promote corneal neovascularization through YTHDF1 recognition and Wnt signaling pathway [28]. On the contrary, the increased expression of FTO and the downregulation of m^{6}A modification were also shown in corneal neovascularization. The activation of FTO/FAK/YTHDF2 signal axis regulates endothelial cell proliferation, migration, and tube formation ability [78]. Specific knockdown of METTL3 in corneal limbal stem cells promotes epithelial cell proliferation and migration after corneal injury, accelerating the recovery rate and the formation of new blood vessels of the cornea, with AHNAK and DDIT4 possibly being its potential molecular targets [79].

2.3.2. Fungal keratitis

In fungal keratitis, METTL3, METTL14 and overall m^{6}A modification levels are notably increased, and significant differences in m^{6}A-modified mRNA exist after fungal keratitis treatment [80,81]. Further research found that METTL3 upregulates pro-inflammatory factors (IL-1β, IL-6, and TNF-α), inflammatory response and downregulates corneal stromal cell activity by targeting the NF-κB/TRAF6 and PI3K/AKT pathway [81,82].

2.4. Cataract

Age-related cataract (ARC) and diabetic cataract are the two main forms of cataract. Cataract pathogenesis includes DNA damage/repair, oxidative stress, proteolysis, ubiquitination, apoptosis, and autophagy [83–86]. An integrated analysis of MeRIP-seq and mRNA sequencing (mRNA-seq) indicates differential expressions of m^{6}A modification-related enzymes in cataract patients with high myopia (METTL14 is upregulated, METTL3, FTO, ALKBH5, YTHDF1, and YTHDF2 are downregulated), and the differentially modified genes are enriched in cellular biology, extracellular matrix formation, and anatomical structure morphogenesis, such as COL6A3, CHI3L1, and PXDN [87].

Among multiple molecular studies, a few circRNAs are associated with cataract, such as circKMT2E, circZNF292, and circHIPK3 [88–90]. Based on MeRIP-seq, one study proposes that the total m^{6}A level is significantly decreased in ARC patients than in control groups, which might act by the addition of ALKBH5, and the differential m^{6}A-modified circRNAs are closely related to oxidative

damage/repair and autophagy [91]. However, *Yang* et al. uncover that upregulated m⁶A modification by METTL3 in the diabetic cataract is responsible for the biological functions of lens epithelial cells, accelerated cell apoptosis, and suppressed proliferation by targeting ICAM-1 [92].

2.5. Glaucoma

The excessive activation of human Tenon's capsule fibroblasts (HTFs) leads to scar formation, which is one of the reasons for the failure of glaucoma filtration surgery. Research shows a high level of m⁶A modification level and METTL3 in TGF-β treated HTFs. Furthermore, significant upregulation of the METTL3/Smad3 signaling axis promotes cell activity, proliferation, and extracellular matrix deposition of HTFs, thereby promoting scar formation and increasing the likelihood of failure after glaucoma surgery [93]. The ganglion cell is the only neuron that collects optical information to the brain, and high intraocular pressure in glaucoma causes dysfunction of ganglion cells, leading to decreased vision or even blindness [94]. Intraocular interference of YTHDF2 increases the number of dendrite branches of ganglion cells and the thickness of the inner plexus layer, and resists the degeneration of ganglion cells and somatic cell loss caused by acute ocular hypertension [95].

2.6. Graves' ophthalmopathy (GO)

As a result of autoimmune disorders, GO frequently concomitantly occurs with Graves' disease in almost 30%–50% of cases [96]. *Zhu* et al. revealed that the total m⁶A level is significantly increased in the groups of GO than controls, and the expressions of m⁶A modification-related enzymes have changed (WTAP, ALKBH5, ELF3, YTHDF2, and YTHDC2 upregulates). Moreover, immune and inflammatory responses are the most changed biological processes between the two groups, which is consistent with pathological mechanisms, such as lymphocyte activation, cytokine-mediated signaling pathway, and regulation of cytokine production [97].

2.7. Primary Sjögren's syndrome (pSS)

A study finds that ALKBH5, RBMX, RBM15B, and YTHDF1 are downregulated in multiple study cohorts of pSS, and KEGG analysis indicates that m⁶A regulators are mainly enriched in immune infiltration and autophagy [98]. Another study also confirms the differential expression of various m⁶A enzymes in the blood and parotid gland samples of pSS patients, which are closely linked with the activation of CD4⁺T cells, CD8⁺T cells, neutrophils, and γδT cells [99].

2.8. Pterygium

Study indicates the total m⁶A level in pterygium significantly decreases compared to normal conjunctival tissue, and METTL3, FTO, and YTHDF1 are also significantly downregulated. DSP, MXRA5, ARHGAP35, TMEM43, and OLFML2A are potential molecular targets
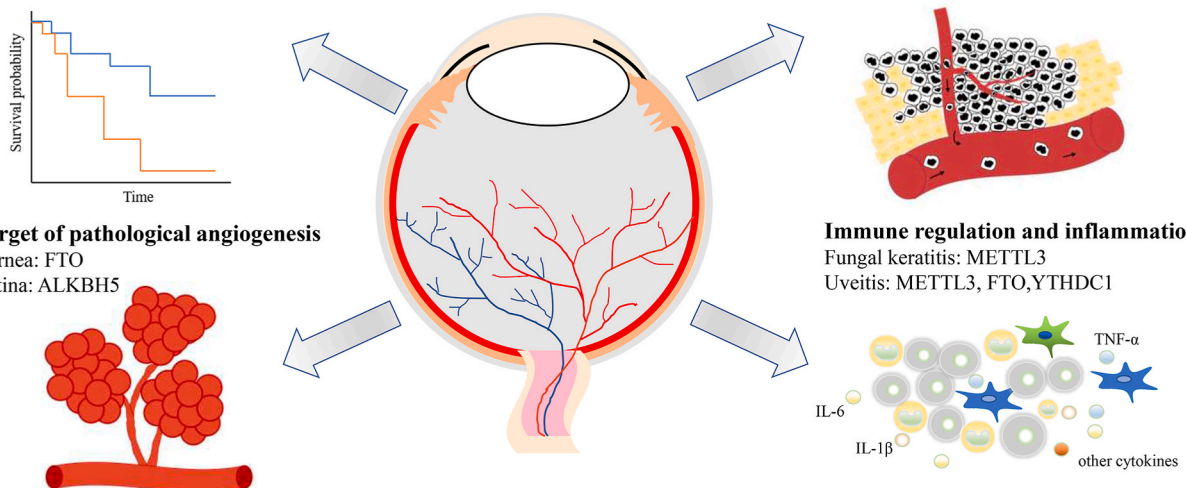

Fig. 3. Future prospects of m⁶A modification in ophthalmic diseases. Abnormal expression of m⁶A modification enzymes has the potential to be regarded as the indicators of prognosis and diagnosis; therapeutic targets of malignancy; targets of angiogenesis-related diseases; as well as the regulation of ocular immune response and inflammation.

for m^{6}A modification by the screening of MeRIP-Seq and RNA-seq [100].

3. Potential diagnosis and treatment strategies associated with m^{6}A modification in ophthalmic diseases

As shown in Fig. 3, m^{6}A modification in ophthalmic treatment targets and clinical applications mainly focus on potential as biomarkers, regulation of neovascularization, tumor progression, and immune and inflammatory responses.

3.1. Drug development targeting m^{6}A modification

Up to now, inhibitors and activators of m^{6}A enzymes have been continuously discovered, including natural ingredients, chemical synthesis, and existing clinical drugs (listed in Table 2). For example, an inhibitor of METTL3, chidamide could enhance crizotinib sensitivity to non-small cell lung cancer by downregulating m^{6}A modification of c-MET [101]. Entacapone decreases the demethylation level of DACT1 and suppresses the progression of osteosarcoma by inhibiting the function of FTO [102]. Moreover, an oral METTL3 inhibitor, sTC-15, has been permitted to conduct clinical trials for acute myeloid leukemia [103]. However, bioavailability, metabolism, selectivity, and local or systemic toxicity are complex and difficult to predict when they are applied to clinical practice, which need further investigation and verification by a wide range of in vivo and in vitro experiments. Although the mechanisms and roles of these drugs on ophthalmic diseases have not been widely studied, they deserve to be deeply explored in the future.

3.2. Potential as prognosis and diagnosis indicators, and therapeutic targets

Previous studies reported that RBM15B, YTHDF1, YTHDF3, and IGF2BP1-2 are closely related to the prognosis of melanoma [65, 66]. Furthermore, IGF2BP3 is closely related to Breslow thickness and clinical stage in cutaneous melanoma [104], thus, the role of IGF2BP3 in ocular melanoma deserves to be further studied. Down expressions of METTL3 and FTO lead to uveitis [54,55]; up-regulation of METTL3 induces fungal keratitis [81]; METTL14 significantly decreases in RP but not in AMD and uveitis patients [45]. Extracellular m^{6}A mainly resources from the degeneration of cellular RNA after cytotoxic stimulation, and accelerates inflammation; moreover, extracellular m^{6}A can be detected in aqueous humor, vitreous humor, plasma, and urine [105], which make them have the potential to be indicators for diagnosis and prognosis, especially for inflammation.

YTHDF2 could recognize and promote the degradation of FTO demethylation-modified PD-1, CXCR4, and SOX10, and inhibit the response of tumor cells to PD-1 immune-blocking therapy [106]. METTL3 regulates retinoblastoma progression, and a previous study reported that SOX4/EZH2/METTL3 signaling pathway contributes to alleviating patients' resistance to temozolomide [107]. These studies illustrate that FTO, METTL3 may be considered as novel therapeutic targets for ocular melanoma and retinoblastoma, respectively.

4. Future research directions and challenges

The functions of m^{6}A modification in ocular diseases have been widely studied, especially in the retinal and choroidal diseases. Pathological mechanisms of retinal diseases mainly focus on the decay function of m^{6}A enzymes on molecular targets, such as YTHDF2 increases the instability of ITGB1 mRNA and facilitates its degradation [41]; and the impact on the translation of transcripts, such as YTHDF1 could enhance the translation of LRP6 and DVL1 [28]. The biological function of retinal related diseases is mainly enriched in the dysfunction of the blood-retinal barrier [28,30,31]. The research emphases of AMD and uveitis are stability regulation of m^{6}A

Table 2
Inhibitors and activators of m^{6}A modification enzymes.

Target	Molecule	Function
METTL3	Baicalin [111], Chidamide [101], Elvitegravir [112], Luteolin, Quercetin, Scutellarin [113], Simvastatin [114], sTC-15 [115], STM2457 [116], UZH1a [117], UZH2 [118]	Inhibition
METTL3/14	photocaging substituent-linked MPCH [119], Fusaric acid [120]	Activation
FTO	Apigenin [121], Betaine [122], Camptothecin and analogs [123], CHTB [124], Clausine E [125], CS1, CS2 [7], Diacerein [126], Entacapone [127], Epigallocatechin gallate [128], FB23 [129], Fluorescein [130], FTO-02, FTO-04 [131], Fusaric acid [120], Hydroxyglutarate [132], Meclofenamic acid [133], MO-I-500 [134], Nafamostat mesilate [135], Naringenin [121], N-CDPCB [124], Quercetin [121], IOX3 [136], Radicicol [124], Rhein [137], Saikosaponin D [138]	Inhibition
FTO	IDH2 [139]	Activation
ALKBH5	ALK-04 [140], Citrate [141], Curcumin [142], Fumarate, Fusaric acid [120], MV1035 [143], IOX1 [144]	Inhibition
IGF2BP1	BTYNB [145]	Inhibition
YTHDC2	Fusaric acid [120]	Activation
YTHDF2	Combination application of resveratrol and curcumin [146], Epigallocatechin gallate [128]	Activation
YTHDF3	Fusaric acid [120]	Activation
–	Cycloleucine [147], Ganetespilb [148], Sulforaphane [149],	Decrease m^{6}A modification level
	Betaine [150]	Upregulate m^{6}A modification level

modification on targets, such as down-expressed YTHDC2 accelerates the degeneration of ASH1L in uveitis [55], and abnormal inflammation response is the most widely explored in these diseases. Regarding pathological conditions of cornea, current research indirections mainly toward angiogenesis and inflammation, and discuss translation changes of targets controlled by m6A modification, such as YTHDF2 could recognize m6A-modified FAK and decrease its mRNA stability [78]. As for ocular tumors, degeneration of targets is explored widely, such as YTHDF3 regulates the translation and decay of CTNNB1 [70]; and investigators link m6A modification to histone modification in ocular melanoma [71,72]. However, several ocular diseases that only explore the changes of m6A modification levels and the significant alternations of enzymes, the deeper mechanisms have not been uncovered, such as GO, pSS, pterygium, cataract, and TON.

m6A modification is a dynamic and reversible modification method. Therefore, when ophthalmic diseases are related to abnormal m6A modification, ideally, the occurrence and progression of the ophthalmic disease can be alleviated or even corrected by changing the methylation level of m6A modification site in the target gene. Hence, a wide range of administration methods is not appropriate, and might instead lead to adverse reactions and pathology in non-lesion areas. In addition to developing novel drugs that can effectively remove or increase m6A modification for target molecules, improving the selectivity and accuracy of drugs against pathological cells and target molecules, and finding brand-new carriers controlling the precise and efficacy for lesion sites are critical strategies in the future. Moreover, the application of single-cell sequencing and metabolomics analysis, regulation networks between m6A modification and non-coding RNA may also help to deeper excavate the underlying mechanisms and new targets.

5. Conclusion

Among numerous modification methods, m6A modification is the most common in eukaryotic cells, and participates in numerous physiological and pathological processes, such as embryonic development, reproduction, tumorigenesis, neurodevelopment, and metabolism [108–110]. The involvement of m6A modification in the normal development of eye structures and the progress of various ocular diseases are summarized above. m6A modification can serve as a powerful candidate target for predicting the occurrence, progression, and adverse prognosis of various eye diseases, and provide ideas for future in-depth research and exploration of m6A modification-related eye diseases. The regulatory mechanisms of m6A enzymes on eye diseases are extraordinarily complex and variable, therefore, the subsequent development of specific drugs and effective, as well as accurate delivery methods deserve further explorations.

Data availability statement

No data was used for the research described in the article.

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CRediT authorship contribution statement

Bingyan Li: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Zicong Wang:** Writing – review & editing. **Haixiang Zhou:** Writing – review & editing. **Jingling Zou:** Writing – review & editing. **Shigeo Yoshida:** Writing – review & editing. **Yedi Zhou:** Conceptualization, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Corresponding author serves as an Associate Editor for Heliyon Clinical Research-Yedi Zhou.

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