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WTAP-mediated N6-methyladenosine mRNA methylation regulates laser-induced macular neovascularization

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Purpose: Neovascular age-related macular degeneration (nAMD) is now a major cause of central vision loss in older adults worldwide. The primary characteristic of nAMD is the formation of macular neovascularization (MNV), which is a pathologic form of angiogenesis. Epigenetics plays a role in multiple pathological physiologic processes. N6-methyladenosine (m6A) modification is the most common, abundant, and reversible modification in eukaryotic mRNAs, and it plays a role in various pathological angiogenesis processes. This study intends to reveal the expression and functions of m6A during the macular neovascularization (MNV) process.

Methods: A laser-induced MNV mouse model was used in this study. m6A quantitative analysis was performed to detect the expression of m6A. Subsequently, the expression of various m6A writers and erasers was detected using quantitative real-time polymerase chain reaction (qRT-PCR) and western blot. Immunohistochemistry was used to detect Wilms' tumor 1-associating protein (WTAP) expression in the MNV lesions. Intravitreal injection of WTAP siRNA in MNV mice to silence the WTAP gene. Hematoxylin and eosin (H&E) were used to determine the thickness and length of the MNV. Fundus fluorescein angiography (FFA) and indocyanine green angiography (ICGA) were examined to measure the leakage area of the MNV. Proliferating cell nuclear antigen (PCNA) expression was detected with a western blot. The mRNA and protein levels of β-catenin were tested with qRT-PCR and western blot.

Results: We found increased m6A modification levels after laser induction compared with the normal control group. Subsequently, the expression of various m6A writers and erasers was detected. The results showed that WTAP increased in the MNV model in mice. After the injection of WTAP siRNA into the vitreous body, the expression of WTAP significantly decreased, subsequently decreasing the m6A modification levels. The width, breadth, and leakage area of MNV damage markedly decreased, and endothelial cell proliferation was inhibited. After laser-induced MNV, the expression of β-catenin increased, and that of β-catenin significantly decreased after WTAP knockout.

Conclusions: In conclusion, this study suggests that WTAP-mediated m6A methylation can regulate pathological angiogenesis during MNV and that WTAP may participate in the formation of MNV through the wingless-related integration site (Wnt) pathway. WTAP may be a potential target for MNV treatment.

The prevalence of neovascular age-related macular degeneration (nAMD) is steadily rising among individuals aged 50 and above worldwide, making it a significant cause of visual impairment in older adults. This upward trend can be attributed to the global phenomenon of population aging [1]. The primary characteristic of nAMD is the formation of macular neovascularization (MNV), which is pathological angiogenesis. These vessels spread from the choroid to the subretinal or retinal pigment epithelium (RPE) layer by disrupting Bruch's membrane, leading to the atrophy of outer photoreceptor cells in the retina and causing a significant effect on visual function [2]. The pathological mechanism of MNV is extremely complicated, and currently, the mechanism of MNV has not been fully elucidated. In the pathological process of MNV, multiple cytokines and cells participate. Research has shown that vascular endothelial growth factor (VEGF) can promote vascular endothelial cell proliferation, induce neovascularization, and affect the chemotaxis of inflammatory cells during neovascularization [3]. Research has also revealed that the role of choroidal endothelial cells (CECs) in the formation of MNV is essential. The proliferation and migration of CECs is caused by the activation of Rac1 in CEC by VEGF, CCL11, or Thy-1 [4].

Currently, the injection of anti-VEGF drugs into the vitreous body is the preferred method for treating MNVs [5]. Other treatment strategies for MNV include laser photocoagulation, transpupillary thermotherapy, photodynamic therapy, and radiation therapy. However, they are just targeted and symptomatic treatments, and side effects are inevitable.

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For example, anti-VEGF drugs can lead to ocular adverse reactions, including retinal damage, lesion scar formation, and choroidal thinning [6]. They have limited clinical efficacy and require repeated injections to maintain therapeutic effects. This is because anti-VEGF agents regulate only one pathway controlling angiogenesis but do not address the underlying causes of abnormal neovascularization [7]. Laser photocoagulation causes damage to the visual field and often leads to scotomas [8]. Therefore, a more thorough investigation of the pathogenic mechanisms of MNV and finding new therapeutic targets requires an urgent solution.

Angiogenesis is typically driven by gene dysregulation. Currently, the mechanism of dysregulation has not been fully elucidated. N6-methyladenosine (m6A) mRNA modification is the predominant and most abundant form of post-transcriptional methylation in eukaryotic cells, accounting for over 50% of all methylated nucleotides [9]. It is present in nearly all categories of RNA, such as mRNA, rRNA, tRNA, snoRNA, miRNA, circRNA, and lncRNA, and plays an important role in various physiologic and pathological biologic processes [10]. The m6A modification interacts with different types of proteins. It affects various aspects of RNA biologic processes, such as translation, transport, subcellular localization, stability, splicing, and degradation, depending on the type of protein with which it interacts [11]. The m6A levels are maintained by the coordinated regulation of m6A methyltransferases (writers) and demethylases (erasers).

Research has shown that m6A modification is involved in a variety of pathological angiogenesis, such as diabetic retinopathy, corneal neovascularization, atherosclerosis, arteriovenous malformation, angiogenesis, and metastasis in various cancers [12]. For example, the downregulation of METTL3 in mouse retinas promotes angiogenesis by increasing the expression of angiogenic genes [13]. In hypoxia-treated breast cancer cells, elevated levels of METTL14 and ALKBH5 lead to the upregulation of VEGF transcription, promoting angiogenesis and cancer metastasis [14]. Another study revealed that fat mass and obesity-associated protein (FTO) promotes corneal neovascularization by inducing the expression of focal adhesion kinase in a mouse model of corneal angiogenesis [15]. The Wilms' tumor 1-associating protein (WTAP) functions as a writer of m6A RNA methyltransferase and plays a crucial role in m6A methylation. Silencing WTAP can inhibit in vitro endothelial cell angiogenesis [16]. The function and regulatory mechanisms of m6A regulators vary due to their subcellular location and different diseases. m6A plays a role in various ocular diseases, including corneal diseases, cataracts, diabetic retinopathy, age-related macular degeneration, proliferative vitreoretinopathy, Graves' disease, uveal melanoma, retinoblastoma, and traumatic optic neuropathy [17,18]. Currently, the role of m6A in the pathogenesis of MNV in vivo remains unclear.

In our study, m6A modification levels in mouse retinal choroidal tissue increased significantly after laser-induced MNV. WTAP silencing can reduce the inhibition of pathological angiogenesis in vivo. Therefore, inhibiting WTAP-mediated m6A modification is an effective measure for alleviating pathological angiogenesis in MNV.

METHODS

Establishing a laser-induced mouse MNV model: The experiment was conducted under the guidelines of the ethics committee of the Affiliated Hospital of Qingdao University (approval number: QYFY WZLL 28352). C57BL/6 mice aged 8 weeks (female; Jinan) were used for processing according to the Association for Research in Vision and Ophthalmology (ARVO) statement. The Lumenis multiple wavelength laser (Dreieich, Germany) slit lamp delivery system was used to perform laser treatment at the four dots around the optic disk. The presence of bubbles observed under the slit lamp during modeling indicates the rupture of the Bruch's membrane. If no bubble formation occurs after laser treatment, if the laser area appears unclear, or if bleeding occurs, such cases will not be included in future analyses. The experimental groups were organized as follows: (1) Normal control group: Healthy mice, no treatment administered. (2) Laser injury group: Mice successfully established with the MNV model. (3) Intravitreal injection (IVI) of PBS group: Immediately after successfully establishing the MNV model in mice, 1.5μl of phosphate-buffered saline (PBS) was injected into the vitreous cavity. (4) IVI Scramble siRNA Group: Immediately after successfully establishing the murine MNV model, Scramble siRNA (1OD siRNA dissolved in 1.5 μl PBS) was injected into the vitreous cavity of the mice. (5) IVI WTAP siRNA Group: Immediately after successfully establishing the murine MNV model, WTAP siRNA (1OD siRNA dissolved in 1.5μl PBS) was injected into the vitreous cavity of the mice.

Quantification of the m6A RNA level: An EpiQuik m6A RNA Methylation Quantification Kit (EpiGentek; P9008–48) was used to determine the m6A RNA modification level in the total RNA samples. According to the product manual, 150 ng of RNA was bound to each well and incubated with the corresponding antibody. Absorbance at 450 nm was measured to calculate the m6A modification level.

RNA isolation and qRT-PCR: The total RNA was obtained from the samples using the RNA iso plus reagent. Following quantification, RNA was reverse transcribed into cDNA, and then use cDNA as a template for PCR amplification. The

Table 1. RT–PCR reactions.

Gene	Primer	Nucleotide sequence (5′→3′)
Mettl3	F	GCAAGCTGCACTTCAGACGAAT
	R	TGCCAGGACTCTCAGAATCAACA
Mettl14	F	ACTGGCATCACTGCGAATGAGA
	R	TCGCAAGCATACTCTCCCAAGG
WTAP	F	GAGTCTGCACGCAGGGAGAA
	R	GGATTTGAGTGGTGCACTCTTGC
FTO	F	TGTCCTCAATGACTCAGACGATGG
	R	AGAACTGCCTCAGCCACTCAA
Alkbh5	F	GCGGTCATCATTCTCAGGAAGA
	R	CTGACAGGCGATCTGAAGCATA

F, forward; R, reverse

mRNA levels of various factors in mice were measured by quantitative real-time polymerase chain reaction (qRT-PCR; Table 1 and Table 2).

Western blot: The protein samples were extracted from the mouse RPE–choroid–sclera complex using a protein extraction reagent (Solarbio, China). SDS–PAGE was used to separate the protein samples on acrylamide, and they were transferred to a polyvinylidene fluoride membrane. The membranes were incubated with the corresponding antibodies. The visualization was achieved using enhanced chemiluminescence (ECL) (Vazyme Biotech, China). ImageJ (National Institutes of Health, Bethesda, MD) was used to quantify the protein blots. The experimental data were analyzed and graphed using GraphPad Prism software (version 9.0; GraphPad Software, La Jolla, CA). A one-way analysis of variance (ANOVA) was used for the statistical analysis among multiple groups. All experiments were repeated three times. The data are expressed as mean ± SD.

Intravitreal injection: WTAP siRNA (22 μg/ul) was injected into the eyes of the mice immediately after laser injury. On the 7th day, all mice were killed. The normal group represented mice without laser injury. The vehicle and scramble siRNA groups represented laser-induced MNV with the injection of PBS and scramble siRNA, respectively. Animals were excluded if they developed lens injury, retinal damage, intraocular hemorrhage, or intraocular inflammation.

Table 2. RT–PCR running conditions.

Step	Temperature	Duration
1	95 °C	30 s
	95 °C	5 s
2	55 °C	30 s
	72 °C	30 s
3	Dissociation stage	

FFA and ICGA: Fundus fluorescein angiography (FFA) and indocyanine green angiography (ICGA) were examined seven days after laser modeling. After 3 min, 2% (weight/volume) fluorescein sodium and 1.25% (weight/volume) ICG were injected into the abdominal cavity. Each mouse was treated using a slit lamp delivery system. Using a digital fundus camera to capture images (No. M10272) ImageJ was used to analyze the extent of leakage in the MNV lesions.

Immunohistochemistry: After 7 days of laser injury, the whole eyes of C57BL/6 mice were taken and encapsulated in the optimal cutting temperature compound (OCT; Sakura Tissue-Tek). A cryostat (Leica, Germany) was used to cut the eyeball into thin 8-um sections at −25°C. The samples were incubated overnight with anti-WTAP antibodies. Diaminobenzidine (DAB) chromogen was added, and staining was terminated for microscopic examination. Images were obtained using a Nikon transmission light microscope.

Histopathology: Each group of eight eyes was encapsulated in an OCT compound, and 8-μm frozen sections were fixed in methanol for 30 min and then stained with hematoxylin and eosin (H&E). A Nikon microscope was used to observe the H&E sections. The MNV lesion area was analyzed using ImageJ.

Statistical analysis: The experimental data were analyzed using GraphPad Prism software (version 9.0). ImageJ was used to quantify the protein blots and measure the MNV lesion length and thickness. All experiments were repeated three times or more. For data involving multiple groups, a one-way ANOVA was used for the statistical analysis. The data are expressed as mean ± SD (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

RESULTS

m6A modification levels increase after laser photocoagulation: To reveal the m6A modification pattern of MNV, we detected the m6A modification level in the total RNA of the C57/B6 mouse model of MNV established after laser injury. Quantitative analysis of m6A showed that compared with the normal state, the m6A level was slightly expressed at 1 and 3 days after laser injury, peaked at 7 days, and then gradually decreased (Figure 1A). These findings imply that m6A modification has a meaningful effect on the pathological progression of MNV.

WTAP expression increases after laser photocoagulation: The modification of m6A is a reversible process catalyzed by m6A writers (methyltransferases) and erasers (demethylases). To identify the regulators of abnormal m6A levels, we investigated the expression of several m6A writers (METTL3, METTL14, WTAP) and erasers (FTO, ALKBH5). The results were obtained using qRT-PCR and western blot analysis. The results showed that, compared with the normal group, the expression level of WTAP substantially increased at the mRNA and protein levels after laser induction (Figure 1B–G), reaching a peak 7 days after laser induction. We also performed immunohistochemical staining of the normal

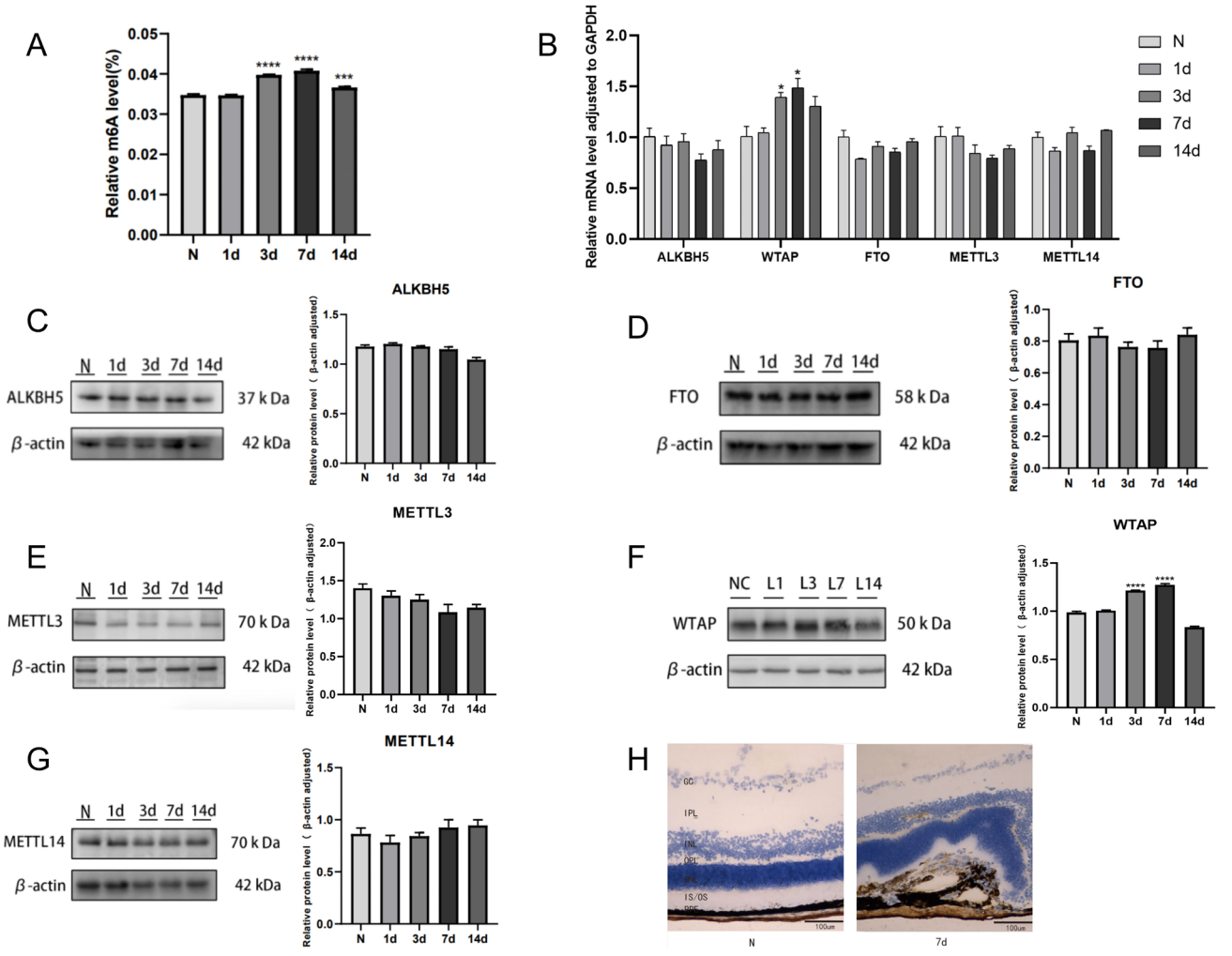

Figure 1. m6A modification levels and WTAP expression increased after laser photocoagulation. **A**: Quantitative analysis of m6A showed a trend in the m6A level compared with the normal control group. **B**: Using qRT-PCR analysis to detect the expression of ALKBH5, WTAP, FTO, METTL3, and METTL14. Western blot was used to detect the expression of ALKBH5 (**C**), FTO (**D**), METTL3 (**E**), WTAP (**F**), and METTL14 (**G**). The statistical analyses were compared with the normal group. **H**: Immunohistochemistry was used to analyze the expression of WTAP in the normal control group and the 7-day group. Scale bar = 100 microns [ganglion cell layer (GC), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), inner segment/outer segment (IS/OS); retinal pigment epithelium (RPE)].

Table 3. The siRNA used in this research.

siRNA	Sequence (5′–3′)	Position
WTAP-siRNA-1	Sense: GGCACGGGAUGAGUUAAUUTT Antisense: AAUUAACUCAUCCCGUGCCTT	302
WTAP-siRNA-2	Sense: GAGUGAAACAGACUUCAAATT Antisense: UUUGAAGUCUGUUUCACUCTT	278
WTAP-siRNA-3	Sense: UCAUGCGGCUAGCAACCAATT Antisense: UUGGUUGCUAGCCGCAUGATT	469

group and the laser-induced group. The results showed that WTAP expression increased in MNV and could have played a role in pathological development (Figure 1H).

The increase in m6A levels after laser induction is mediated by WTAP: To further determine the role of WTAP in MNV pathology and to investigate whether WTAP is a direct regulatory factor of m6A methylation in the pathological process of MNV, we immediately injected WTAP siRNA into the vitreous body after successfully establishing a mouse MNV model. We designed three WTAP siRNAs (Table 3), with WTAP siRNA-1 exhibiting the strongest silencing effect (Figure 2A) and were used in subsequent experiments. QRT-PCR and western blot analysis were used to detect WTAP expression levels in the retinal choroidal complex. The results showed that WTAP expression significantly decreased after the injection of WTAP siRNA compared with the 7-day group, the vehicle (PBS) group, and the interfering scramble siRNA group (Figure 2B–D). The m6A quantitative analysis showed a significant decrease in m6A levels after WTAP silencing (Figure 2E). These results indicate that WTAP regulates m6A methylation in the pathogenesis of MNV.

WTAP siRNA reduces MNV leakage and decreases MNV lesion areas: To determine the role of WTAP in the pathogenesis of MNV, we conducted HE staining, which showed that the thickness and length of MNV were greatly reduced after the injection of WTAP siRNA into the vitreous body (Figure 3A). The thickness of the MNV lesions was measured and analyzed using ImageJ. The results of FFA and ICGA showed that the leakage area of MNV markedly decreased after WTAP knockout compared with the 7-day group, the vehicle (PBS) group, and the scramble siRNA group (Figure 3B–F).

WTAP siRNA inhibits the proliferation of CECs: To further confirm whether WTAP siRNA inhibits the proliferation of CECs, we detected the expression of the proliferating cell nuclear antigen (PCNA). Western blot analysis showed that the expression of PCNA increased after laser induction and decreased after silencing WTAP (Figure 4A–D). The above results indicate that WTAP can regulate CECs, and injecting WTAP siRNA into the vitreous body inhibits CEC proliferation.

WTAP may regulate the MNV pathological process through the Wnt signaling pathway: To investigate how WTAP regulates the MNV pathological process, qRT-PCR and western blot were used to determine the expression of β-catenin. The results showed that the expression of β-catenin increased after laser induction and that the expression of β-catenin was inhibited by WTAP siRNA (Figure 5A-C). This indicates that WTAP may regulate the MNV pathological process through the wingless-related integration site (Wnt) signaling pathway.

DISCUSSION

The occurrence and development of MNV is a pathological process of angiogenesis involving various cells and cytokines [19]. In this process, the permeability of blood vessels increases, leading to the degradation of the vessel wall and the basal membrane, followed by cell migration and extracellular matrix (ECM) deposition, thickening of endothelial cells and the formation of lumens, creating a series of processes to form new blood vessels [20]. Previous studies have shown that many pathogenic factors influence the pathological progression of MNV. However, the mechanism of MNV is still not fully understood. Thus, there is still a need for new targets in the study of MNV generation mechanisms.

Various epigenetic factors regulate vascular development. Studies have indicated that the dysregulation of epigenetic modifications, such as RNA methylation, DNA methylation, and histone modification, can regulate angiogenesis due to their dynamic and reversible nature [21].

The m6A mRNA modification was originally discovered in the 1970s and is the most common and reversible modification in eukaryotic genes [22]. m6A modification occurs in the consensus sequence DRACH (D = A, G, U; R = A, G; H = A, C, U), with an average of three m6A modifications per mRNA in this motif. The global m6A level is maintained by the synergistic regulation of the m6A methyltransferases (writers) and demethylases (erasers). Methyltransferases (writers) include METTL3, METTL14, METTL16, WTAP,

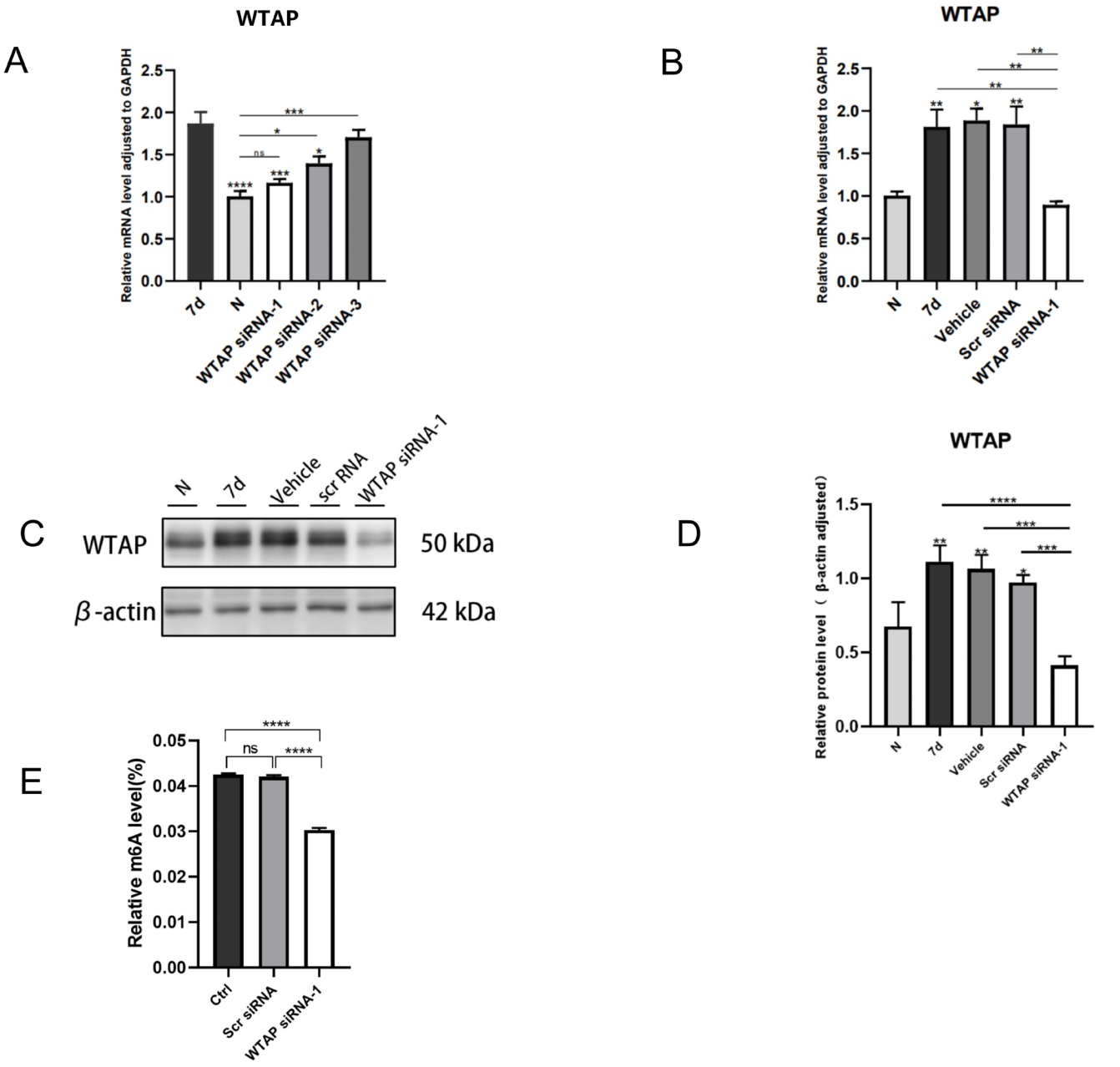

Figure 2. WTAP siRNA downregulates WTAP expression and m6A modification levels. **A**: After injecting three WTAP siRNAs into the vitreous body, qRT-PCR analysis was used to detect the expression of WTAP. QRT-PCR analysis (**B**) and protein immunoblotting (**C** and **D**) were used to measure WTAP expression after intraocular injection of WTAP siRNA. **E**: Quantitative analysis suggests that compared to the 7-day group, the m6A level was reduced after WTAP siRNA was injected into the vitreous body.

KIAA1429, RBM15, and ZFP217, while RNA demethylases (erasers) include FTO and ALKBH5 [23]. Previous research has indicated that aberrations in m6A methylation may be involved in various pathological changes, including inflammation, microorganism infection, autoimmune disease, degeneration, senescence, epithelial–mesenchymal transition, angiogenesis, fibrosis, and tumorigenesis [10].

Neovascularization is a complicated process involving the recognition of biologic signals related to hypoxia, inflammation, and metabolic dysregulation; the secretion of proteases, pro-angiogenic factors, cytokines, and their binding

to corresponding receptors; endothelial cell proliferation; selective degradation of the extracellular matrix; dissolution of the vascular basement membrane; and the establishment of luminal structures [24]. In recent years, studies have shown that abnormal m6A modification is involved in the pathogenic angiogenesis of various diseases. For example, in hypoxia-treated breast cancer cells, the levels of METTL14 and ALKBH5 are elevated, leading to the upregulation of the VEGF transcript and promoting angiogenesis and cancer metastasis [14]. Hypoxia-induced downregulation of METTL3 in human umbilical vein endothelial cells

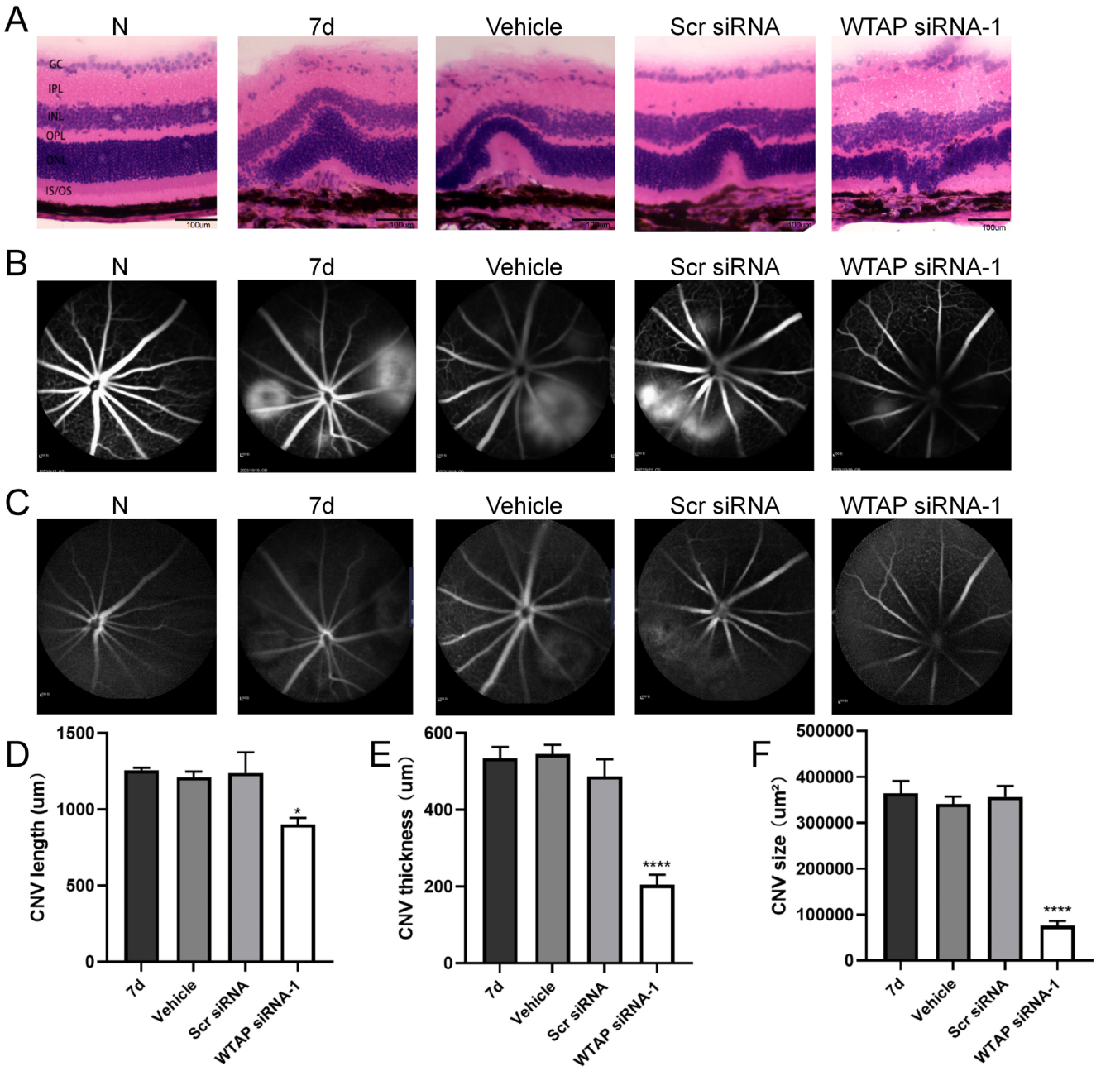

Figure 3. WTAP siRNA reduces the area of MNV lesions and alleviates MNV leakage. **A**: H&E staining showed the size of the MNV lesions in each group. **B**, **C**: Compared with the 7-day group after laser induction, the fluorescein leakage around the laser spot after the intravitreal injection of WTAP siRNA was weaker. Scale bar = 100 microns. Statistical analysis of the MNV length (**D**), thickness (**E**), and leakage area (**F**) in the 7-day group, the vehicle group, the scramble siRNA group, and the WTAP siRNA group compared with the 7-day group.

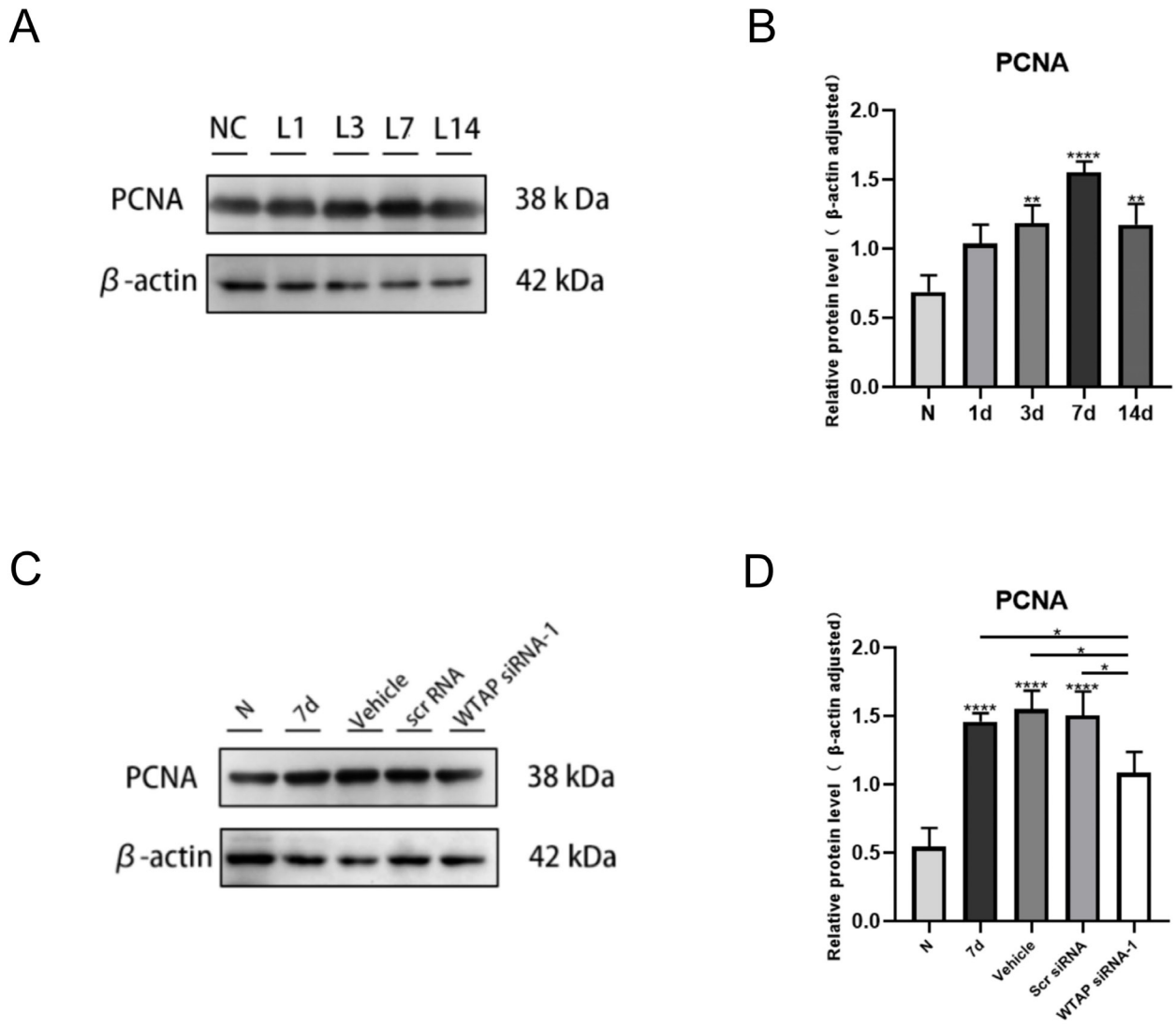

Figure 4. WTAP siRNA inhibits the proliferation of CECs in MNV. **A**: Using western blot to detect PCNA levels before and after laser induction. **B**: Statistical analysis revealed a trend in PCNA expression compared with the normal group. **C**: Using western blot to detect the protein level of PCNA in the control group and the WTAP siRNA group injected into the vitreous body. **D**: Statistical analysis showing the trend of PCNA expression.

(HUVECs) promotes angiogenesis by upregulating the expression of angiogenic genes [13].

Another study revealed that FTO promotes corneal neovascularization by inducing the upregulation of the focal adhesion kinase in a mouse corneal neovascularization model [15]. The function and regulatory mechanisms of m6A regulators vary due to their subcellular location and different diseases. In different diseases or pathogenic mechanisms, m6A regulators may play opposite roles. For example, FTO has been shown to exhibit anti-angiogenic effects on intrahepatic cholangiocarcinoma but has been shown to promote angiogenesis in diabetic retinopathy [25,26]. These findings indicate that m6A modification plays a crucial role in maintaining normal vascular physiology and endothelial cell function, and that its disruption can lead to disease occurrence. Therefore, further studies are needed to investigate the role of m6A in MNV pathology.

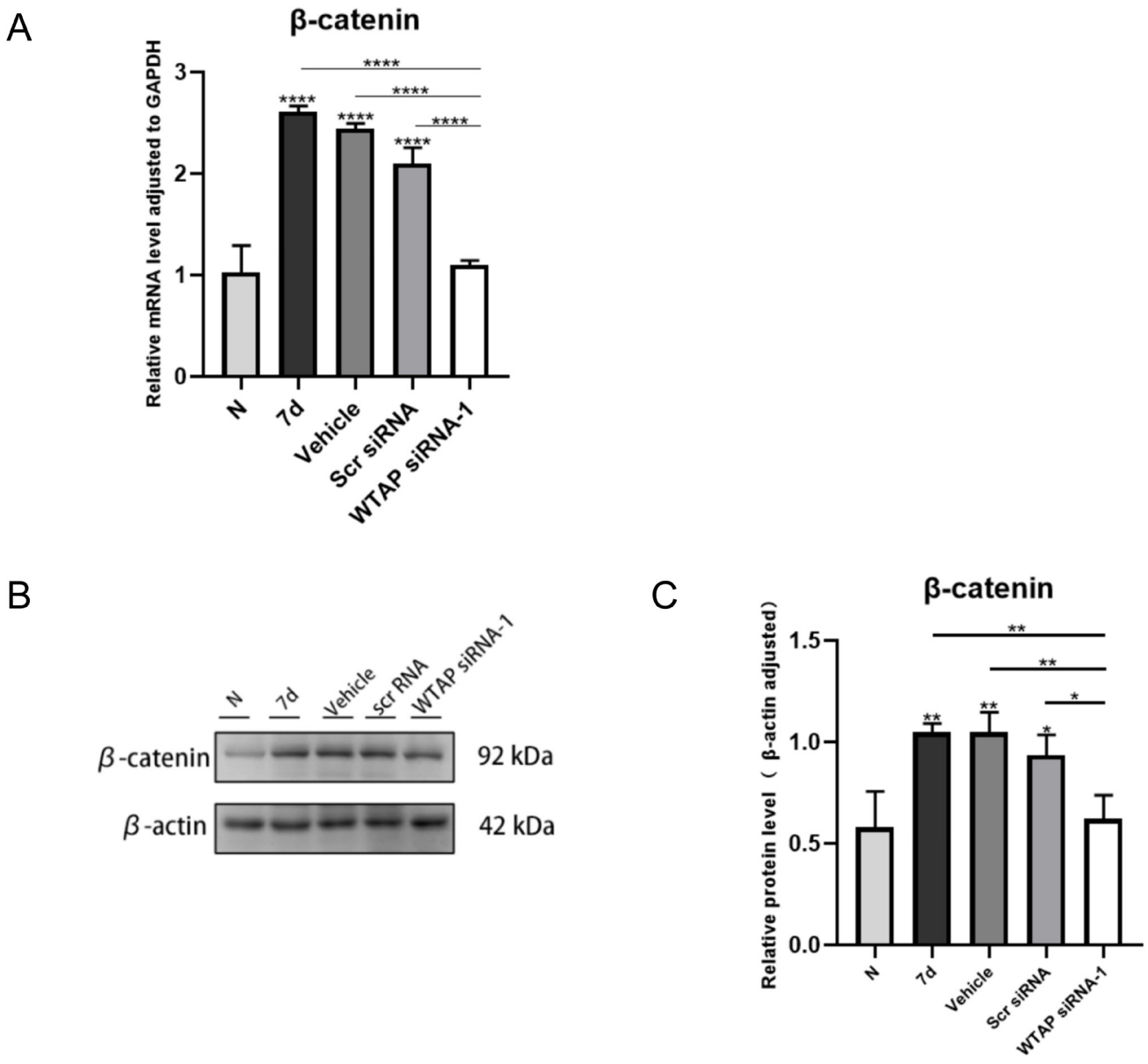

Figure 5. WTAP may affect the MNV pathological process through the Wnt pathway. **A**: QRT-PCR analysis showed a significant decrease in β-catenin expression after intravitreal injection of WTAP siRNA. **B**: Using western blot to measure the expression of β-catenin. **C**: Statistical analysis showing the trend of β-catenin expression.

This study used laser injury to establish a mouse model of MNV. The laser penetrated Bruch's membrane, disrupting the choroid and breaking down the barrier between the retina and the choroid. After laser damage, the region of the laser spot causes hypoxia and inflammatory reactions due to vascular damage, resulting in the formation of neovascularization. Furthermore, due to the destruction of Bruch's membrane, neovascularization develops under the retina [27]. We demonstrated the regulation of pathological angiogenesis through m6A methylation. In this study, the m6A modification level increased in laser-induced MNV mice compared with normal mice. The expressions of the writers and erasers of m6A modification were detected, and the results showed that, after laser induction, the expression of WTAP significantly increased. In contrast, the expressions of the other m6A writers (METTL3 and METTL14) and erasers (FTO and ALKBH5) did not change significantly, leading to increased levels of m6A modification.

WTAP is involved in RNA metabolism and is a regulatory subunit of the m6A methyltransferase complex [28]. Research has shown that the WTAP level is downregulated in arteriovenous malformations and that arteriovenous malformations lead to capillary malformations by destabilizing desmoplakin [29]. Another study has shown that WTAP affects the recruitment of macrophages in endothelial cells and that the translation regulation of HIF-1a mediated by m6A affects the secretion of VEGF/A/C/D macrophages. Both pathways regulate WTAP during angiogenesis and lymphangiogenesis during MNV [16]. The WTAP gene was silenced by intravitreal injection of WTAP siRNA in mice to further investigate the role of WTAP in the pathological process of MNV.

The results showed that, compared with the MNV mice induced by laser alone, after silencing WTAP, the expression level of WTAP significantly decreased, the breadth and width of MNV damage markedly decreased, and the area of leakage was significantly reduced. Therefore, according to our results, silencing WTAP can reduce m6A levels and alleviate pathological angiogenesis. The results also indicate that after WTAP gene silencing, the expression of PCNA correspondingly decreased. This suggests that WTAP may inhibit neovascularization by inhibiting the proliferation of CECs in MNV mice. Due to various complex signaling mechanisms in the process of angiogenesis, targeting m6A modification by modulating WTAP levels is a promising strategy for treating MNV.

The Wnt signaling pathway regulates endothelial cell behavior to control vascular morphogenesis [30]. Wnt ligands mediate the vascular endothelial cell function and homeostasis of the vascular environment through short-range paracrine. Abnormal activation of the Wnt signaling pathway is an important cause of pathological neovascularization, especially in diabetic retinopathy, wet age-related macular degeneration, and retinopathy of prematurity [31]. The Wnt signaling pathway has been reported to be suppressed by the WTAP–WT1–TBL1 axis. WT1 functions as a suppressor of the Wnt signaling pathway. The WTAP/WT1 complex is formed through the interaction between WTAP and WT1. Reduced levels of the WTAP protein result in the liberation of WT1, which triggers the transduction of beta-like protein-1 (TBL1) and leads to a decrease in beta-catenin levels [32]. Therefore, it is worth exploring whether WTAP affects the Wnt signaling pathway in endothelial cells.

Previous studies have shown that silencing or the overexpression of WTAP decreases or increases the protein levels of β-catenin, affecting the Wnt signaling pathway. In our study, we found that β-catenin expression was elevated in MNV lesions, and that β-catenin expression was significantly inhibited by silencing the WTAP gene. Therefore, we speculate that WTAP may play a role in the formation of MNV by affecting the Wnt pathway. Nevertheless, our study has some limitations. The precise mechanism underlying the involvement of WTAP in the pathological progression of MNV necessitates further investigation.

In conclusion, upregulation of WTAP expression in MNV lesions increases m6A modification levels. The involvement of WTAP is pivotal in the pathological progression of MNV. Mechanistically, WTAP may exert its pro-angiogenic role through the abnormal activation of the Wnt signaling pathway. The clinical applicability of WTAP as a novel molecular target for the treatment of nAMD requires further detailed investigation.

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