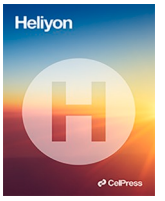
Heliyon 10 (2024) e27893

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Tocilizumab attenuates choroidal neovascularization by regulating macrophage polarization through the IL-6R/STAT3/VEGF pathway

Yuanyuan Tu [a], Yang Guo [a], Haotian Sun [a], Yuting Zhang [a], Qiaoyun Wang [b], Yiqian Xu [b], Laiqing Xie [b,**], Manhui Zhu [a,*]

[a] Department of Ophthalmology, Lixiang Eye Hospital of Soochow University, Suzhou, Jiangsu, China
[b] Department of Ophthalmology, The Second Affiliated Hospital of Soochow University, Jiangsu, China

ARTICLE INFO

Keywords:
Choroidal neovascularization
TCZ
IL-6
Macrophage polarization

ABSTRACT

Globally, age-related macular degeneration (AMD) is the leading cause of irreversible visual impairment. Up to 80% of severe vision loss is caused by AMD, which is characterized by the development of choroidal neovascularization (CNV). Uncertainty exists regarding the precise pathophysiological mechanisms of CNV. It has been suggested that the interleukin (IL) IL-6/IL-6R signaling pathway is crucial in the progression of CNV. Tocilizumab (TCZ), a monoclonal antibody, binds to soluble and membrane-bound IL-6R and competitively inhibits IL-6 downstream signaling. Previous research has demonstrated that TCZ promotes several roles related to inflammation and neovascularization. However, the effects of TCZ on CNV and the underlying mechanism are still unknown. This study found that TCZ administration decreased the area and leakage of CNV lesions in the mice model of laser-induced CNV. Additionally, results demonstrated that TCZ promotes the expression of iNOS, CCL-3, CCL-5, TNF-α and inhibits the expression of Arg-1, IL-10, YM-1 and CD206. Furthermore, TCZ treatment inhibited the signal transducer and activator of transcription (STAT) STAT3/vascular endothelial growth factor (VEGF) pathway, which was activated after CNV formation. Colivelin, a STAT3 agonist, reversed the inhibitory effects of TCZ on CNV formation and macrophage polarization. In a mouse model of laser-induced CNV, our findings demonstrated that TCZ attenuated CNV formation and inhibited the leakage of CNV lesions by regulating macrophage polarization via inhibiting the STAT3/VEGF axis. TCZ is the potential therapeutic strategy for CNV.

1. Introduction

The most common worldwide factor contributing to irreversible vision loss is age-related macular degeneration (AMD) [1]. Neovascular AMD ("wet" AMD) and Geographic atrophy ("dry" AMD) are the two primary categories for AMD clinically. Neovascular AMD accounts for 15%–20% of all cases of this condition, while wet AMD accounts for up to 80% of severe vision loss [2].

Neovascular AMD is characterized by choroidal neovascularization (CNV). The development of abnormal vessels causes exudation,

* Corresponding author.
** Corresponding author.
E-mail addresses: xielaiqing@suda.edu.cn (L. Xie), zhumanhuieye@126.com (M. Zhu).

https://doi.org/10.1016/j.heliyon.2024.e27893
Received 13 October 2023; Received in revised form 7 March 2024; Accepted 7 March 2024
Available online 14 March 2024

hemorrhage, retinal edema, pigment epithelium separation, fibrotic scar formation, and vision loss. The abnormal vessels grow in the outer and subretinal regions after penetrating the Bruch's membrane. The most common form of treatment for CNV is anti-vascular endothelial growth factor (VEGF), which has made significant progress in the field and significantly altered the prognosis of the disease [3]. However, anti-VEGF treatments can be expensive and ineffective in certain patients [4]. The pathogenesis of CNV needs to be studied since it could help develop more effective CNV treatment plans.

We do not fully understand the precise pathophysiological mechanisms underlying CNV. According to previous research, the progression of CNV is accelerated by infiltrating macrophages, which has revealed that monocytes in the circulation go to CNV lesions and undergo macrophage differentiation [5]. Traditionally activated (M1 type) and alternatively activated (M2 type) macrophage subtypes can exist simultaneously. Specific M1 macrophage markers, such as inducible nitric oxide synthase (iNOS) and CCL-3, have anti-angiogenic effects [6]. The pathogenesis of CNV is aided by M2 macrophages bearing particular markers, such as Arg-1 and YM-1 [7]. A higher ratio of M2/M1 macrophage was discovered in the CNV mouse model and the aqueous humor of patients with CNV [8,9]. When the vitreous cavities of mice are injected with M2 macrophages, the formation of CNV lesions is exacerbated, whereas M1 macrophages improved condition [9]. As a result, it is conceivable that inhibiting M2 macrophage polarization could be used as a potential treatment for CNV.

Interleukin (IL) IL-6 has reportedly been shown to promote macrophage M2 polarization in recent decades [10]. In obese mice fed a high-fat diet, IL-6 promotes macrophage M2 polarization and adipose tissue proliferation [11]. By secreting different cytokines, the cancer-associated adipocytes (CAAs), created from adipocytes close to breast cancer tissues, promote tumorigenesis and the spread of breast cancer. Breast cancer could advance due to CAAs being able to secrete large amounts of IL-6, which activates the signal transducer and activator of transcription (STAT) STAT3 signaling pathway and causes macrophage M2 polarization [12]. Compared to the healthy controls, IL-6 levels were elevated in the retinal pigment epithelium (RPE) choroid tissues of the laser-induced mouse CNV model [13]. The intracellular signaling pathway is activated when IL-6 binds to IL-6R and subsequently connects with co-receptor gp130. For instance, the IL-6/STAT3/VEGF pathway has been linked to macrophage activation and M2 polarization [14], and IL-6 increases macrophage polarization towards the M2-type through STAT3 activation [10]. Based on previous research, we proposed that IL-6 promotes macrophage M2 polarization by binding to the IL-6R and activating the STAT3/VEGF signaling pathway, contributing to the formation of CNV.

Tocilizumab (TCZ), a monoclonal antibody, blocks IL-6 downstream signaling [15] by binding to soluble and membrane-bound IL-6R. A subconjunctival injection of TCZ prevents VEGF expression in the cornea [16] and corneal neovascularization brought on by alkali burns. TCZ prevents the oncogene *MCT-1* [17] from causing cancer invasion and macrophage M2-type polarization. However, how TCZ affects the progression of CNV and macrophage polarization is unclear.

In conclusion, this study aimed to investigate more about how IL-6/IL-6R and its inhibitor, TCZ, influence macrophage polarization and CNV progression.

2. Materials and methods

2.1. Animals

Male wild-type C57BL/6, aged 6–8 weeks, were randomized into five groups, including the normal group, CNV 7d, CNV 7d + PBS, CNV 7d + TCZ (0.01 μg/μL), CNV 7d+ TCZ(0.02 μg/μL). The mice were kept in a typical housing arrangement with free access to water and food during the 12-h light/dark cycle. As previously reported [18], the laser-induced mouse CNV model was used. Briefly stated mice were given an intraperitoneal injection of 2% pentobarbital (Tocris, USA; 2% w/v) to induce anesthesia. Following pupil dilation, a laser spot with a diameter of 50 μm was fired at the periphery of the optic disc of the mouse in the directions of 3, 6, 9, and 12 o'clock using a PASCAL diode ophthalmic laser system (Topcon, USA). As a sign of the breakthrough of Bruch's membrane, the appearance of a bubble at the location of the photocoagulation point was used.

2.2. Quantitative real-time polymerase chain reaction (qRT-PCR)

Using Trizol agents (Invitrogen, USA), the total RNA from RPE-choroid complexes was extracted. First-Strand complementary DNA (cDNA) Synthesis Kit was used to reverse transcribe 1 μg of total RNA from each sample into cDNA. The SYBR Premix Kit (Takara) was used for qRT-PCR following the manufacturers' instructions. Supplemental Table 1 displays the target gene sequences, and the $2^{-\triangle\triangle Ct}$ method was used to determine the relative mRNA expression. GAPDH was used as an internal reference gene.

2.3. Western blots

RIPA buffer with proteinase and phosphatase inhibitors was used to extract the total protein from RPE-choroid complexes. The protein from each sample was divided into equal quantities using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride filter (PVDF) membranes. The membranes were then treated at 4 °C for an overnight period with primary antibodies, including STAT3, p-STAT3, VEGF, IL-6R, and GAPDH. Supplemental Table 2 displays specific information about the antibodies. The membranes were then treated with the corresponding secondary antibodies and three washes. The internal control was GAPDH. A Bio-Rad Gel Doc EZ Imager (Bio-Rad) was used to capture images of the gels.

2.4. Hematoxylin and eosin (H&E) staining

Mice in each group were handled appropriately, after which they were sacrificed and had their eyes removed. As previously described, Retina-RPE-choroid paraffin-embedded sections and H&E staining were carried out [19]. The sections, which passed through the center of each lesion with the largest cross-sectional area, were used to assess the size of CNV lesions.

2.5. Fundus fluorescein angiography (FFA)

On the seventh day following laser photocoagulation, mice were anesthetized with 2% pentobarbital before receiving an intraperitoneal injection of 2% fluorescein sodium (0.3 ml, Akron Pharmaceuticals, USA). Five min following the injection, late angiograms were taken. Finally, two experts evaluated the level of CNV leakage using the previously published CNV Leakage Rating Criteria [20].

2.6. Immunofluorescence of choroidal flat mounts

The mice were killed 7 d after laser photocoagulation, and the RPE-choroid complexes were surgically separated and examined under a microscope. For 2 h, RPE-choroid complexes were fixed with 4% paraformaldehyde (Solarbio Lifesciences, China). They were then permeabilized with 0.1% Triton X-100 (Beyotime, China) for 30 min and blocked with 1% bovine serum albumin (Sigma, MA, USA). They were then incubated with primary antibodies against phalloidin, isolectin B4, F4/80, iNOS, Arg-1, and VEGF at 4 °C overnight. RPE-choroid complexes were washed and then incubated with secondary antibodies. Pictures were captured using a confocal microscope (Leica, Germany).

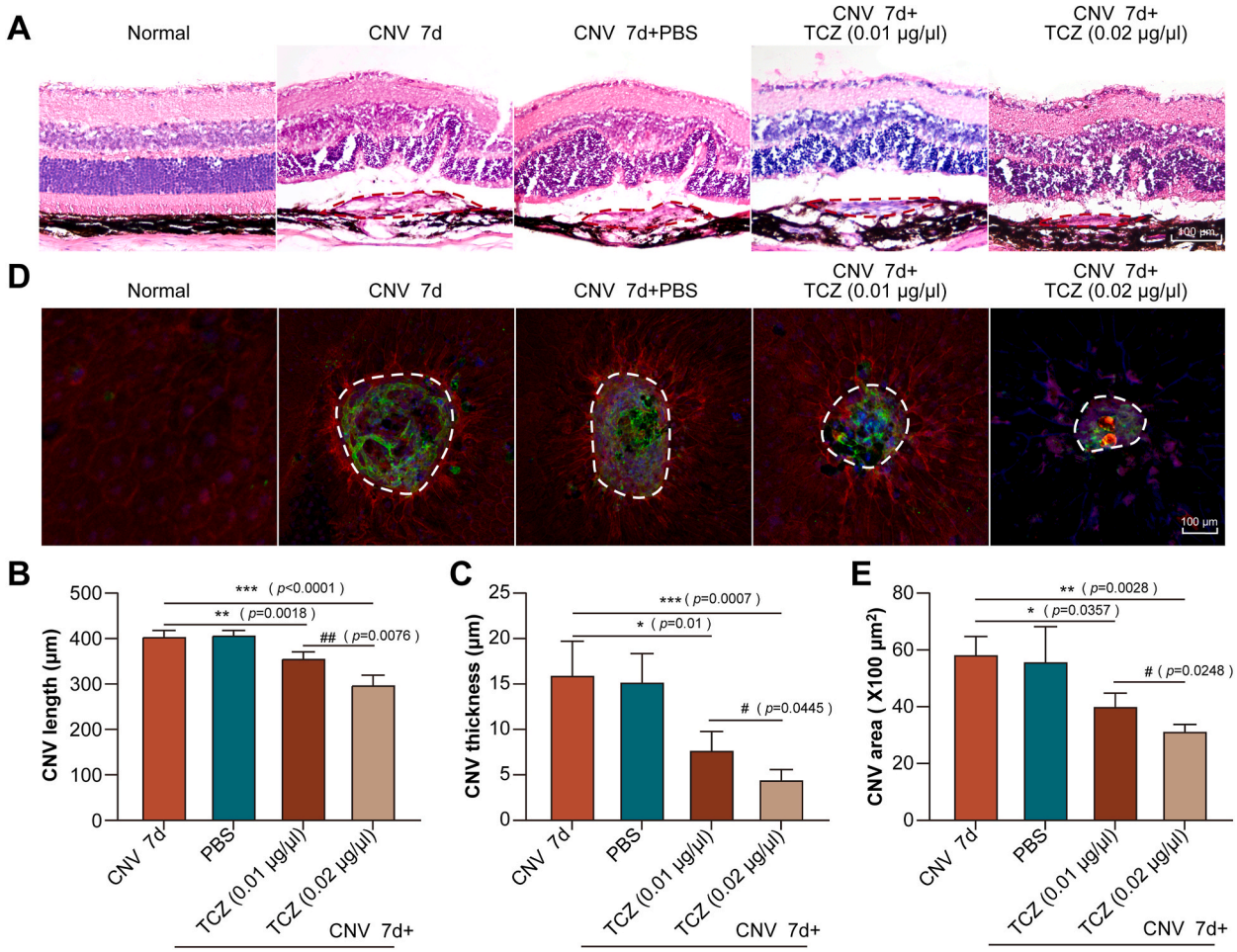


Fig. 1. (A). The length and thickness of CNV lesions were measured using Hematoxylin & Eosin staining. Scale bar: 100 μm. (B). Quantification of the length of CNV lesion. (C). Quantification of the thickness of CNV lesion. (D). Phalloidin (red) and isolectin B4 (green) were used to mark the choroid flat mounts, and a confocal laser microscope was used to capture the images. Scale bar: 100 μm. (E). Measurement of the CNV lesion area. *P < 0.05 vs. CNV 7 d group, #P < 0.05 vs. TCZ (0.1 μg/μL). n = 8/group. Data are expressed as Mean ± SD.

2.7. Statistical analysis

All data are expressed as mean ± standard deviation. A significant difference between the two groups was examined using Student's t-test. Analysis of variance and the post-hoc Bonferroni test were used to determine the statistical significance of differences between several groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. The effects of tocilizumab (TCZ) on the formation of choroidal neovascularization (CNV) lesions in a laser-induced mouse model

The role of TCZ in CNV was examined using a laser-induced mouse CNV model. The size of CNV lesions was analyzed using H&E staining. The findings demonstrated that TCZ decreased the length and thickness of CNV lesions, with 0.02 μg/μL being the most effective concentration (Fig. 1A-C). On the seventh day following laser photocoagulation, mice were euthanized, and the choroidal tissues were made into flat-mounting RPE-choroid complexes. We used Phalloidin and IB4 to label CNV lesions. The high concentration of 0.02 μg/μL demonstrated a more significant inhibitory effect on CNV formation, and similarly, the area of CNV lesions was significantly inhibited by TCZ treatment (Fig. 1D-E).

3.2. The effects of TCZ on the leakage of CNV lesions in a laser-induced mouse model

We used FFA to determine how TCZ affected the pathological vascular leakage of CNV. The results showed that the mice in the TCZ treatment group had fewer and smaller areas of vascular leakage, and the concentration of 0.02 μg/μL was most effective and chosen for further experiments (Fig. 2A–B).

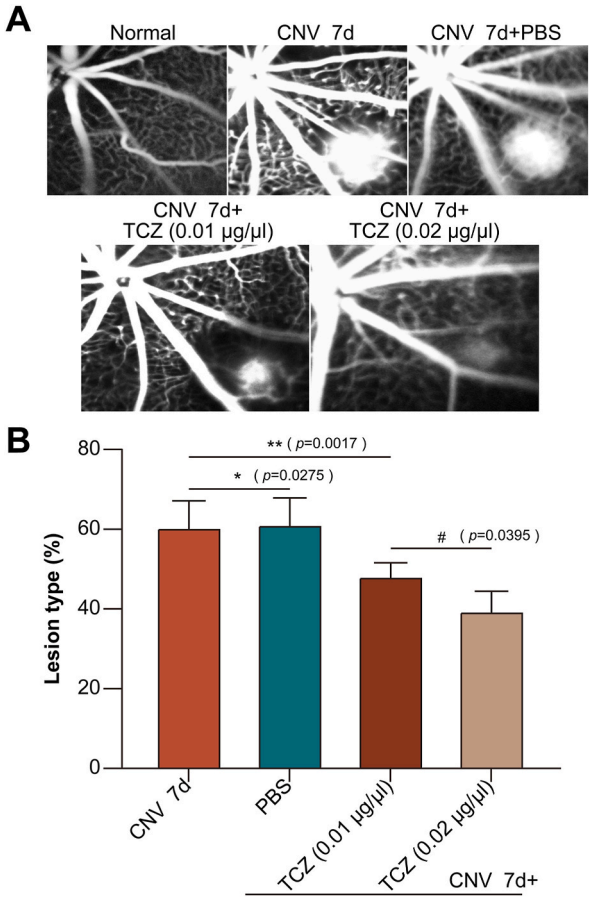


Fig. 2. The effects of TCZ on the leakage of CNV lesions in a laser-induced mouse model.
(A) The Fundus fluorescein angiography (FFA) assay was used to find the leakage of CNV lesions. After 2% intraperitoneal fluorescein sodium was administered to the mice under general anesthesia, late-phase angiograms were collected 5 min later. (B) Quantification of CNV lesion grade. *P < 0.05 vs. CNV 7 d group, #P < 0.05 vs. TCZ (0.1 μg/μL). n = 8/group. Data are expressed as Mean ± SD.

3.3. The effects of TCZ on macrophage M1/M2 polarization

The RPE-choroid complexes after laser photocoagulation had higher mRNA levels of M1-type macrophage markers (iNOS, CCL-3, CCL-5 and TNF-α) (Fig. 3A–D) and M2-type macrophage markers (Arg-1, IL-10, YM-1 and CD206) (Fig. 3E–H). TCZ administration increased the expression of M1-type macrophage markers while it decreased the mRNA levels of M2 macrophage-related markers. The RPE-choroid complexes marked with iNOS and Arg-1 responded similarly to qRT-PCR (Fig. 3I–J). These findings imply that TCZ inhibits the formation of CNV by promoting macrophage polarization toward the anti-angiogenic M1-type and preventing their polarization toward the pro-angiogenic M2-type.

3.4. TCZ inhibits the activation of STAT3/VEGF axis

The IL-6R inhibitor TCZ is used to identify the role of STAT3/VEGF signaling pathway in the progression of CNV. After successfully modeling CNV, we discovered that p-STAT3 and VEGF protein expression increased while being reversed by TCZ treatment (Fig. 4A–E). These findings suggested that the STAT3/VEGF signaling pathway may be involved in the pathogenesis of CNV, and TCZ therapy counteracted the effects.

3.5. The effects of colivelin on IL-6R/STAT3/VEGF signaling pathway

In the following investigations, a STAT3 agonist, Colivelin, was used to examine the STAT3/VEGF signaling pathway that TCZ regulates in more detail. Western blot analysis revealed that IL-6R protein expression remained constant (Fig. 5A–B). However, compared to the CNV + TCZ group, the expression of p-STAT3 and VEGF was significantly elevated in the CNV + TCZ + Colivelin group (Fig. 5C–E). Colivelin abolished the effects of TCZ on CNV formation.

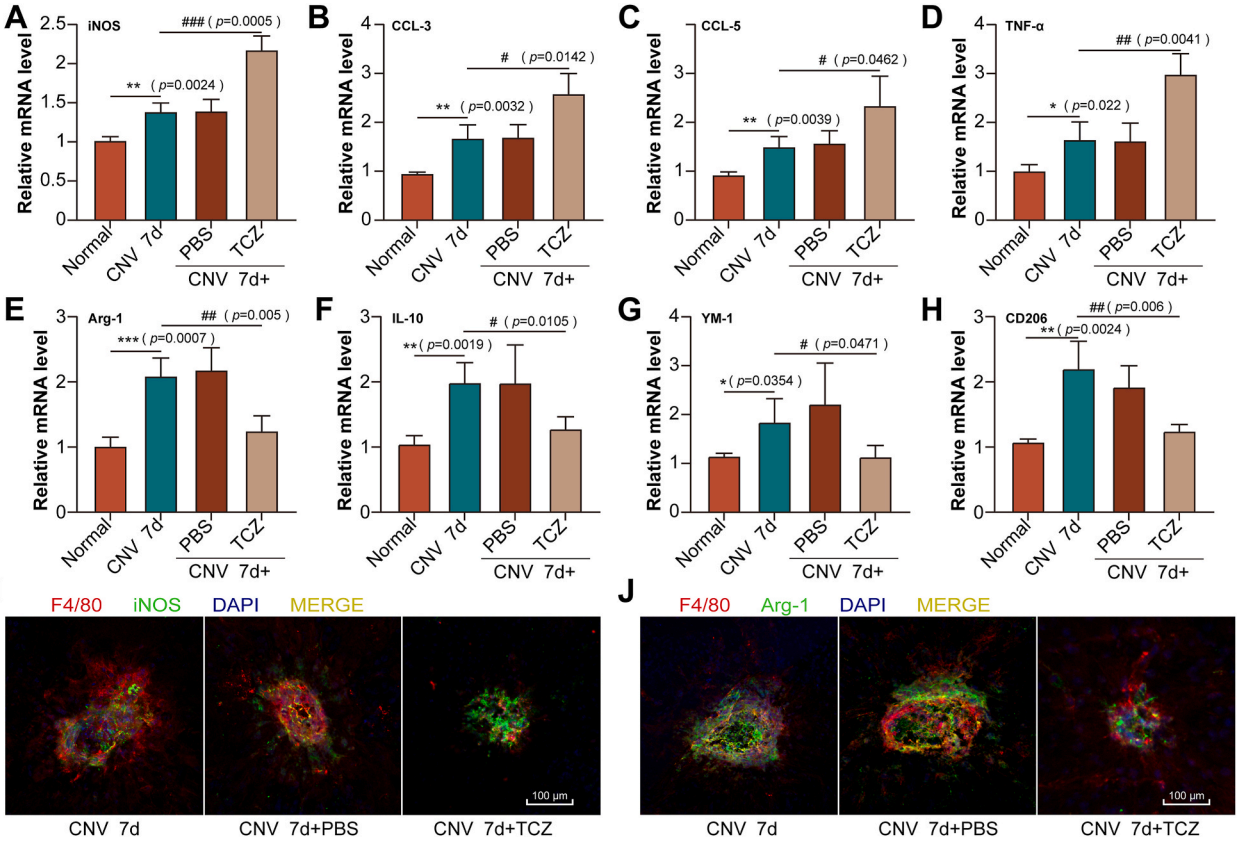


Fig. 3. The effects of TCZ on macrophage M1/M2 polarization.
Quantitative real-time polymerase chain reaction was used to identify the mRNA levels of iNOS (A), CCL-3 (B), CCL-5 (C), TNF-α (D), Arg-1 (E), IL-10 (F), YM-1 (G) and CD206 (H) were detected using RPE-choroid complexes. (I). Images were taken with a confocal laser microscope and marked with F4/80 (red) and iNOS (green) on the flat mounts of the choroid. Scale bar: 100 μm. (J). F4/80 (red) and Arg-1 (green) are used to mark the flat mounts of the choroid. Scale bar: 100 μm *P < 0.05 vs. Normal group, #P < 0.05 vs. CNV 7 d, n = 8/group. Data are expressed as Mean ± SD.

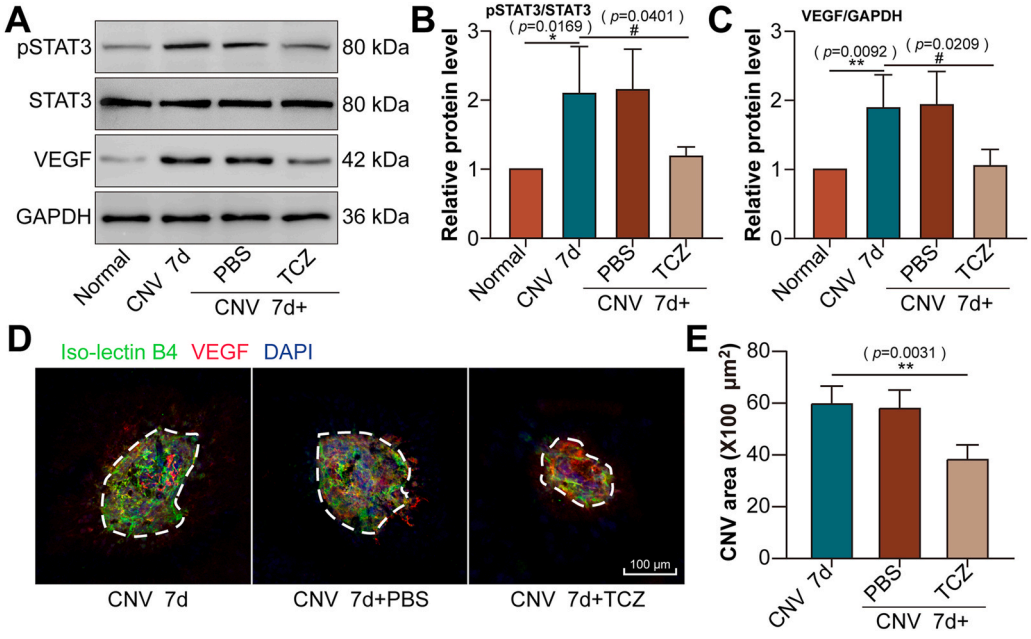


Fig. 4. TCZ inhibits the activation of STAT3/VEGF axis.
(A). A Western blot analysis found p-STAT3, STAT3, and VEGF proteins. (B). Quantification of p-STAT3/STAT3. (C). Quantification of VEGF. (D). Iso-lectin B4 (green) and VEGF (red) were used to label the flat mounts of the choroid, Scale bar: 100 μm. (E). Quantification of choroid neovascularization (CNV) area. *P < 0.05 vs. normal group, #P < 0.05 vs. CNV 7 d, n = 6/group. Data are expressed as Mean ± SD.

3.6. The effects of colivelin on macrophage M1/M2 polarization

Using the STAT3 agonist Colivelin, the impact of the STAT3/VEGF pathway on CNV macrophage polarization was examined. When compared to the CNV + TCZ group, the M1 macrophage marker (iNOS, CCL-3, CCL-5 and TNF-α) showed a decrease in mRNA expression, while the M2 macrophage marker (Arg-1, IL-10, YM-1 and CD206) showed an increase in the CNV + TCZ + Colivelin group (Fig. 6A–H). Immunofluorescence detection of choroidal flat mounts produced the same outcomes (Fig. 6I–J).

3.7. The effects of colivelin on the formation of CNV lesions in a laser-induced mouse model

Next, we looked into how STAT3/VEGF signaling affected CNV. The size of CNV lesions was measured using H&E staining. The findings showed that the length and thickness of CNV lesions were reduced in the CNV + TCZ + Colivelin group (Fig. 7A–C). Then, using choroidal flat mount immunofluorescence, the CNV area was found. Colivelin, a STAT3 agonist, increased the area of the CNV lesion compared to the CNV + TCZ group (Fig. 7D–E).

4. Discussion

Aqueous humor and serum levels of IL-6, a potent proinflammatory cytokine, are elevated in patients with AMD [21,22]. The volume of macula within 6 mm positively correlated with the expression level IL-6 in aqueous humor. The maximum thickness of the macula and the volume of the macula within 1, 3, and 6 mm were shown to be substantially correlated with the expression levels of IL-6 when the expression of IL-6 in the aqueous humor of patients with CNV was further screened [23]. The mRNA and protein expression of IL-6 was elevated in mice's retina and RPE-choroid complexes with CNV, according to research by Kim et al. [24] and Izumi-Nagai et al. [25]. Blocking of IL-6 receptors or significantly reducing IL-6 levels prevented CNV formation. Additionally, *in vivo* and *in vitro*, IL-6 receptor neutralization reduced the expression of molecules associated with inflammation and the infiltration of macrophages at the CNV 25. Accordingly, IL-6 may be a possible target for CNV therapy. In line with earlier research, we discovered in our study that laser treatment increased the expression of IL-6 in the RPE-choroid complexes of mice. Additionally, TCZ administration decreased the polarization of macrophage M2 in CNV mice models by blocking the IL-6/IL-6R signaling pathway.

A monoclonal antibody called TCZ prevents IL-6 from binding to IL-6R [26]. TCZ has been used to treat various cases of ocular inflammation and neovascularization. After acute retinal necrosis and noninfectious uveitis [27,28], macular edema is treated with TCZ. Comparing TCZ to anti-TNF-α agents, Leclercq et al. found that the complete response of macular edema brought on by uveitis was improved [29]. After receiving TCZ treatment, the visual acuity of two patients with birdshot chorioretinopathy who had failed to respond to conventional immunosuppressive drugs, such as interferon α2a and anti-TNFα agents, improved by 80% [28]. Furthermore, it has been shown that TCZ plays a role in preventing ocular neovascularization [16,30]. Injecting TCZ subconjunctivally in New

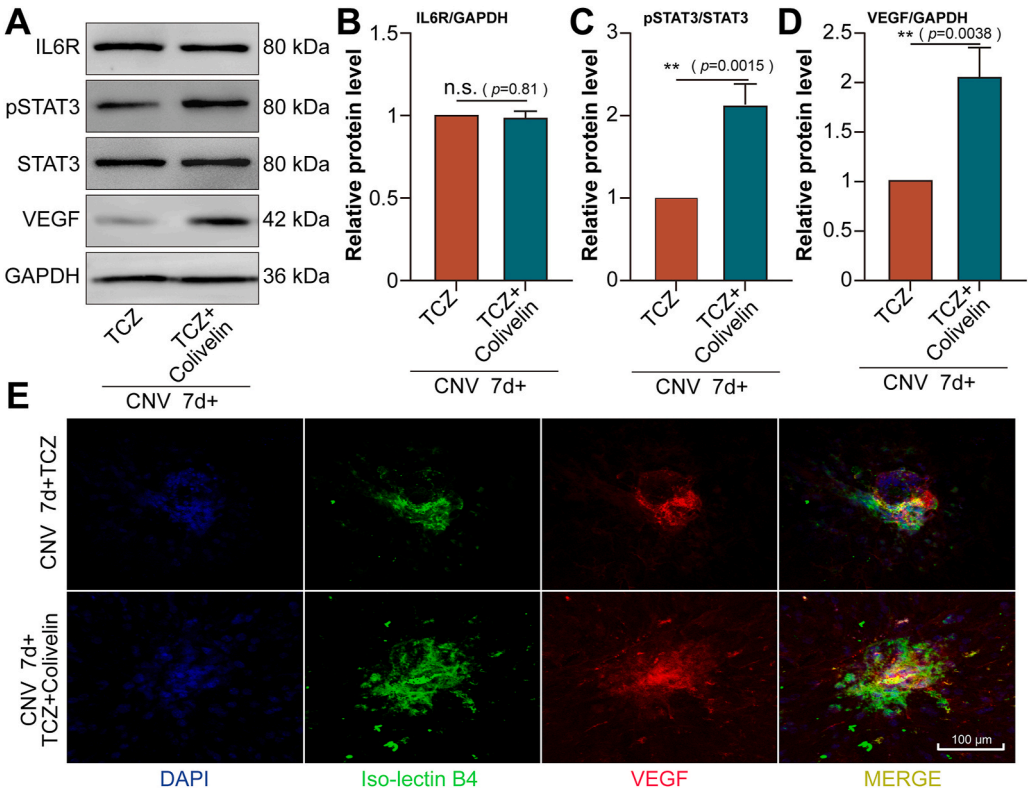


Fig. 5. The effects of colivelin on IL-6R/STAT3/VEGF signaling pathway.
(A). Western blot analysis was used to detect the protein of IL-6R, p-STAT3, STAT3, and VEGF. (B). Quantification of IL-6R. (C). p-STAT3/STAT3. (D). Quantification of VEGF. (E). Iso-lectein B4 (green) and VEGF (red) were used to mark the flat mounts of the choroid, Scale bar: 100 μm *P < 0.05 vs. TCZ group, n = 6/group. Data are expressed as Mean ± SD.

Zealand white rabbits prevented corneal neovascularization and had effects comparable to bevacizumab. Compared to the control group, immunohistochemical analysis revealed that VEGF expression decreased [30]. However, the specific mechanisms were not investigated. In our research, we discovered that the administration of TCZ decreased both the formation of CNV and the expression of VEGF, and the activation of the STAT3 signaling pathway caused this effect.

CNV causes visual loss in the wet form of AMD, also known as vascular AMD. Numerous studies have noted the presence of macrophages with CNV, and immune activation has been demonstrated to play a significant role in the pathogenesis of CNV [31]. The number of IL-6^{+} macrophages was increased in the peripheral blood and the eyes after laser injury [13]. The macrophage-depleted mice displayed decreased vascularity and cellularity in the CNV lesions and a smaller area of CNV compared to the control group [32]. Furthermore, CL2MDP-lip administration 2 days prior to laser injury was more advantageous than immediately after. The CNV volume was correlated to the number of invading macrophages and the level of VEGF protein [33]. These reports verified that macrophages have promoting effects on the formation of CNV.

Mouse CNV models and CNV patients' bodily fluids showed a higher M2/M1 ratio [8,9]. When M2 macrophages were injected into mice's eyes, CNV lesions' formation worsened, but M1 macrophages improved them [9]. M2 macrophages may more significantly influence the pathophysiology of CNV. Macrophages with one phenotype can easily change to another when the microenvironment is altered [34]. Zhiping Liu et al. reported that in laser-induced mouse CNV models, the necrotic RPE cells promoted the key glycolytic activator 6-phosphofructo-2-kinasefructose-2,6-bisphosphatase-3 (PFKFB3)-induced glycolysis of macrophages, leading to the induction of M1/M2 markers and proangiogenic cytokines, which eventually resulting in the reprogramming of macrophages towards to an angiogenic phenotype to facilitate the development of CNV [35]. The hyperglycolytic macrophages/microglia also play an important role in retinal angiogenic niche [36]. A research from Droho, Steven et al. [37] showed that CD11c $^{+}$ monocyte-derived macrophages are pro-angiogenic and pro-glycolytic. CD11c $^{+}$ pro-angiogenic macrophages differentially express higher Arg1 and Chil3/YM-1, which is in agreement with our results. However, this pro-angiogenic subset does not differentially express more Mrc1/CD206 or IL-10 in disagreement with our results, this may be due to the fact that we assayed the entire RPE-choroid complex. However, our study did not focus on the glycolytic process in macrophages. Previous studies have reported that STAT3 was involved in glycolysis [38–40]. STAT3 inhibition attenuated the expression of PFKFB3 and the hyperglycolysis of HMrSV5 cells [39]. STAT3 activated by IL-6 promote the expression of PFKFB3, resulting in enhanced glycolysis in oncogenesis [41]. Based on the above research results, we speculated that activated STAT3 in the laser-induced mouse CNV model may also be involved in the formation of CNV by regulating the glycolysis process. Further experiments will be performed to verify our speculation in the future.

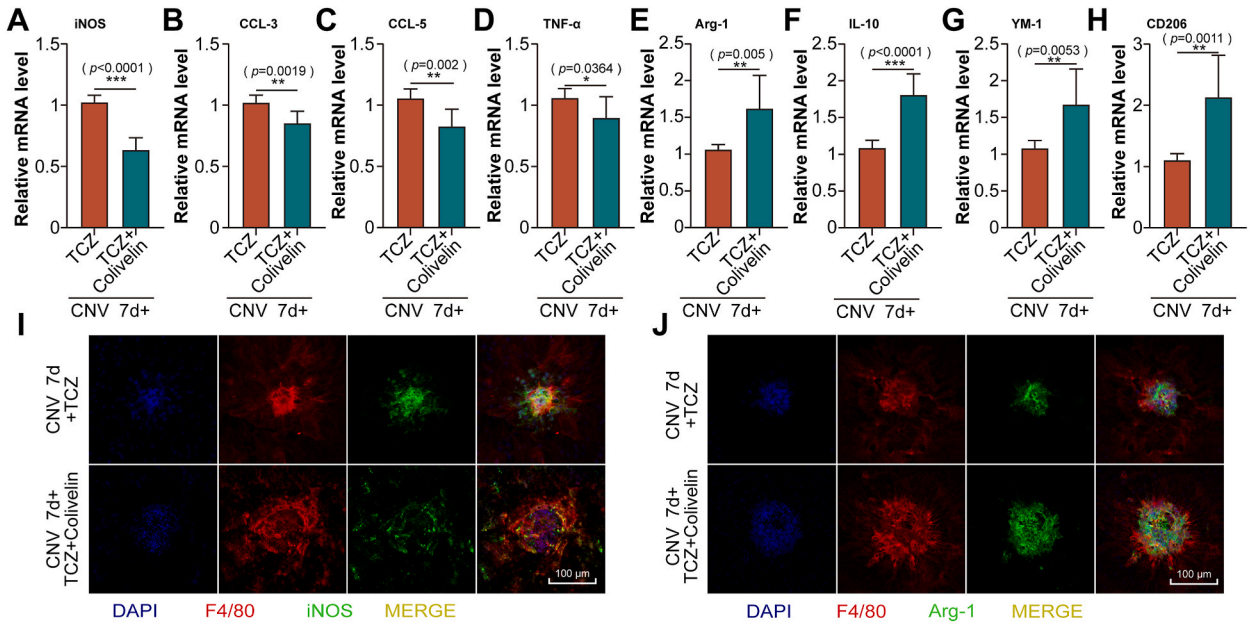


Fig. 6. The effects of colivelin on macrophage M1/M2 polarization.
Quantitative real-time polymerase chain reaction (qRT-PCR) was used to identify the mRNA levels of iNOS (A), CCL-3 (B), CCL-5 (C), TNF-α (D), Arg-1 (E), IL-10 (F), YM-1 (G) and CD206 (H) in RPE-choroid complexes. (I). Images were taken using a confocal laser microscope and marked with F4/80 (red) and iNOS (green) on the flat mounts of the choroid. Scale bar: 100 μm. (J). F4/80 (red) and Arg-1 (green) were used to mark the flat mounts of choroid, Scale bar: 100 μm *P $<$ 0.05 vs. TCZ group, n = 6/group. Data are expressed as Mean ± SD.

The complex mechanisms underlying macrophage polarization include a significant function for the STAT3 signaling pathway. By triggering the STAT3 signaling pathway, tumor cell-derived leukemia inhibitory factor promotes macrophage M2 polarization [42]. By regulating M2 macrophage polarization and invasion, targeting STAT3 is an efficient therapeutic approach for treating corneal neovascularization [14]. Inhibiting the IL-6/STAT3 signaling pathway reduces the progression of hepatocellular carcinoma by reducing M2-type macrophage polarization [10]. Our study demonstrated that mice with CNV have increased STAT3/VEGF pathway activation and M2 macrophage polarization in RPE-choroid complex. STAT3 activation and M2 macrophage polarization were reduced after TCZ treatment. STAT3 agonists abolished the promotion of macrophage M2 polarization by TCZ. In addition to impacting macrophage polarization, inhibiting the STAT3/VEGF signaling pathway reduces CNV, which was consistent with previous studies [43].

In conclusion, the current study showed that, in a mice model of laser-induced CNV, TCZ controlled macrophage polarization from M2-type to M1-type by blocking the STAT3/VEGF axis, thereby reducing CNV formation and preventing lesion leakage. A potential therapy approach for CNV could be TCZ.

Ethics declarations

This study was reviewed and approved by Ethics Committee of Lixiang Eye Hospital of Soochow university, with the approval number SLER2021410.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Yuanyuan Tu: Writing – original draft, Software, Resources, Investigation, Funding acquisition, Data curation. **Yang Guo:** Software, Resources, Investigation, Data curation. **Haotian Sun:** Investigation. **Yuting Zhang:** Resources, Methodology, Investigation. **Qiaoyun Wang:** Visualization, Software, Resources, Methodology, Investigation. **Yiqian Xu:** Writing – original draft. **Laiqing Xie:** Writing – review & editing, Visualization, Methodology, Funding acquisition. **Manhui Zhu:** Writing – review & editing, Software, Project administration, Methodology, Investigation, Funding acquisition.

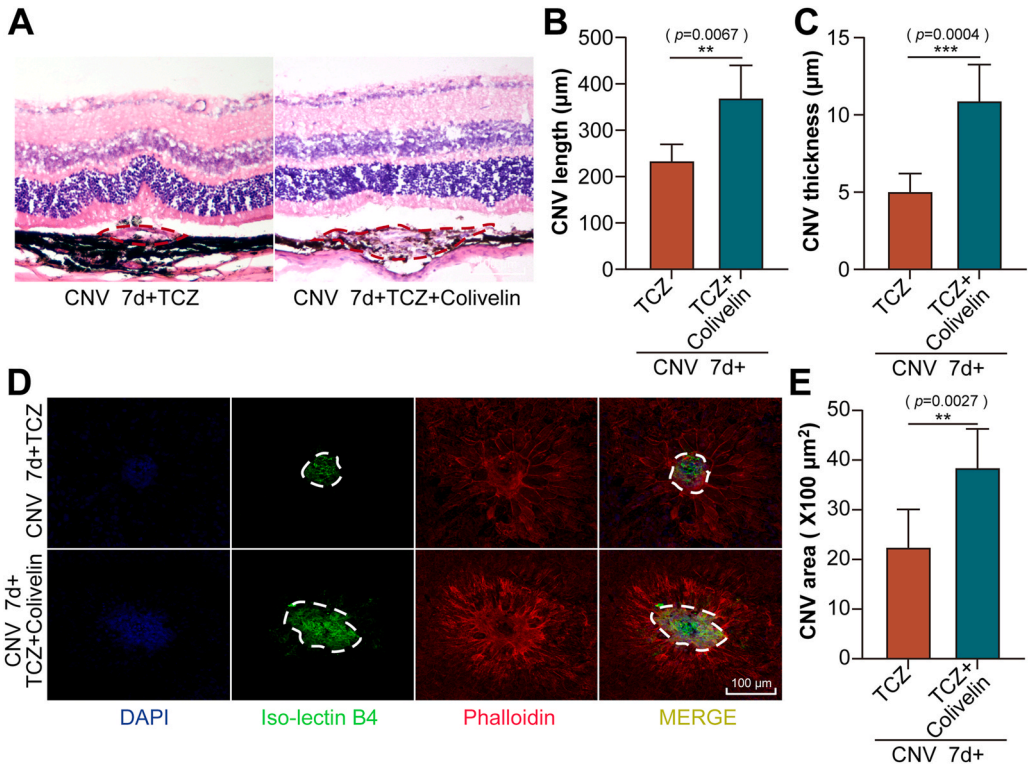


Fig. 7. The effects of colivelin on the formation of CNV lesions in a laser-induced mouse model.
(A) The length and thickness of CNV lesions were measured using Hematoxylin & Eosin staining. Scale bar: 100 μm. (B) Quantification of the length of CNV lesion. (C) Quantification of the thickness of CNV lesion. (D) Isolectin B4 (green) and Phalloidin (red) were used to mark the flat mounts of choroid, Scale bar: 100 μm. (E) Measurement of CNV lesion area. *P < 0.05 vs. TCZ group, n = 5/group. Data are expressed as Mean ± SD.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The National Natural Science Foundation of China (No. 82000911, No. 82201219), Gusu Health Talent Program Project in Suzhou [No. (2022)192], Medical research project derived from Health Commission of Jiangsu Province (No. M2020053) supported this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27893.

References

[1] W.M. Al-Zamil, S.A. Yassin, Recent developments in age-related macular degeneration: a review, Clin. Interv. Aging 12 (2017) 1313–1330, https://doi.org/10.2147/CIA.S143508.
[2] C.J. Thomas, R.G. Mirza, M.K. Gill, Age-related macular degeneration, Med. Clin. 105 (3) (2021) 473–491, https://doi.org/10.1016/j.mcna.2021.01.003.
[3] F. Ricci, F. Bandello, P. Navarra, G. Staurenghi, M. Stumpp, M. Zarbin, Neovascular age-related macular degeneration: therapeutic management and new-upcoming approaches, Int. J. Mol. Sci. 21 (21) (2020), https://doi.org/10.3390/ijms21218242.
[4] G.J. Jaffe, T.A. Ciulla, A.P. Ciardella, F. Devin, P.U. Dugel, C.M. Eandi, H. Masonson, J. Mones, J.A. Pearlman, M. Quaranta-El Maftouhi, et al., Dual antagonism of PDGF and VEGF in neovascular age-related macular degeneration: a phase IIb, multicenter, randomized controlled trial, Ophthalmology 124 (2) (2017) 224–234, https://doi.org/10.1016/j.ophtha.2016.10.010.
[5] M. Wu, Y. Liu, H. Zhang, M. Lian, J. Chen, H. Jiang, Y. Xu, G. Shan, S. Wu, Intravenous injection of l-aspartic acid beta-hydroxamate attenuates choroidal neovascularization via anti-VEGF and anti-inflammation, Exp. Eye Res. 182 (2019) 93–100, https://doi.org/10.1016/j.exer.2019.03.018.

[6] Y. Xu, K. Cui, J. Li, X. Tang, J. Lin, X. Lu, R. Huang, B. Yang, Y. Shi, D. Ye, et al., Melatonin attenuates choroidal neovascularization by regulating macrophage/microglia polarization via inhibition of RhoA/ROCK signaling pathway, J. Pineal Res. 69 (1) (2020) e12660, https://doi.org/10.1111/jpi.12660.

[7] P. Zhang, H. Wang, X. Luo, H. Liu, B. Lu, T. Li, S. Yang, Q. Gu, B. Li, F. Wang, et al., MicroRNA-155 inhibits polarization of macrophages to M2-type and suppresses choroidal neovascularization, Inflammation 41 (1) (2018) 143–153, https://doi.org/10.1007/s10753-017-0672-8.

[8] Y. Shen, L.F. Dong, R.M. Zhou, J. Yao, Y.C. Song, H. Yang, Q. Jiang, B. Yan, Role of long non-coding RNA MIAT in proliferation, apoptosis and migration of lens epithelial cells: a clinical and in vitro study, J. Cell Mol. Med. 20 (3) (2016) 537–548, https://doi.org/10.1111/jcmm.12755.

[9] S. Zandi, S. Nakao, K.H. Chun, P. Fiorina, D. Sun, R. Arita, M. Zhao, E. Kim, O. Schueller, S. Campbell, et al., ROCK-isoform-specific polarization of macrophages associated with age-related macular degeneration, Cell Rep. 10 (7) (2015) 1173–1186, https://doi.org/10.1016/j.celrep.2015.01.050.

[10] Z. Yin, T. Ma, Y. Lin, X. Lu, C. Zhang, S. Chen, Z. Jian, IL-6/STAT3 pathway intermediates M1/M2 macrophage polarization during the development of hepatocellular carcinoma, J. Cell. Biochem. 119 (11) (2018) 9419–9432, https://doi.org/10.1002/jcb.27259.

[11] J. Braune, U. Weyer, C. Hobusch, J. Mauer, J.C. Bruning, I. Bechmann, M. Gericke, IL-6 regulates M2 polarization and local proliferation of adipose tissue macrophages in obesity, J. Immunol. 198 (7) (2017) 2927–2934, https://doi.org/10.4049/jimmunol.1600476.

[12] C. Zhao, N. Zeng, X. Zhou, Y. Tan, Y. Wang, J. Zhang, Y. Wu, Q. Zhang, CAA-derived IL-6 induced M2 macrophage polarization by activating STAT3, BMC Cancer 23 (1) (2023) 392, https://doi.org/10.1186/s12885-023-10826-1.

[13] S. Droho, C.M. Cuda, H. Perlman, J.A. Lavine, Macrophage-derived interleukin-6 is necessary and sufficient for choroidal angiogenesis, Sci. Rep. 11 (1) (2021) 18084, https://doi.org/10.1038/s41598-021-97522-x.

[14] Y. Wang, Y. Gao, Y. Huang, Y. Pan, Y. Yu, Y. Zhou, S.S. Wan, Y.N. Yang, The potential protective effects of miR-497 on corneal neovascularization are mediated via macrophage through the IL-6/STAT3/VEGF signaling pathway, Int. Immunopharm. 96 (2021) 107745, https://doi.org/10.1016/j.intimp.2021.107745.

[15] L. Yang, J. Guo, N. Yu, Y. Liu, H. Song, J. Niu, Y. Gu, Tocilizumab mimotope alleviates kidney injury and fibrosis by inhibiting IL-6 signaling and ferroptosis in UUO model, Life Sci. (2020) 261, https://doi.org/10.1016/j.lfs.2020.118487.

[16] E.S. Sari, A. Yazici, H. Aksit, A. Yay, G. Sahin, O. Yildiz, S.S. Ermis, K. Seyrek, B. Yalcin, Inhibitory effect of sub-conjunctival tocilizumab on alkali burn induced corneal neovascularization in rats, Curr. Eye Res. 40 (1) (2015) 48–55, https://doi.org/10.3109/02713683.2014.914541.

[17] Y.S. Weng, H.Y. Tseng, Y.A. Chen, P.C. Shen, A.T. Al Haq, L.M. Chen, Y.C. Tung, H.L. Hsu, MCT-1/miR-34a/IL-6/IL-6R signaling axis promotes EMT progression, cancer stemness and M2 macrophage polarization in triple-negative breast cancer, Mol. Cancer 18 (1) (2019) 42, https://doi.org/10.1186/s12943-019-0988-0.

[18] M. Zhu, L. Jiang, Y. Yuan, L. Chen, X. Liu, J. Liang, Q. Zhu, D. Ding, E. Song, Intravitreal Ets1 siRNA alleviates choroidal neovascularization in a mouse model of age-related macular degeneration, Cell Tissue Res. 376 (3) (2019) 341–351, https://doi.org/10.1007/s00441-019-03001-1.

[19] Y. Tu, M. Zhu, Z. Wang, K. Wang, L. Chen, W. Liu, Q. Shi, Q. Zhao, Y. Sun, X. Wang, et al., Melatonin inhibits Muller cell activation and pro-inflammatory cytokine production via upregulating the MEG3/miR-204/Sirt1 axis in experimental diabetic retinopathy, J. Cell. Physiol. (2020), https://doi.org/10.1002/jcp.29716.

[20] N. Lara-Castillo, S. Zandi, S. Nakao, Y. Ito, K. Noda, H. She, M. Ahmed, S. Frimmel, Z. Ablonczy, A. Hafezi-Moghadam, Atrial natriuretic peptide reduces vascular leakage and choroidal neovascularization, Am. J. Pathol. 175 (6) (2009) 2343–2350, https://doi.org/10.2353/ajpath.2009.090439.

[21] T. Mimura, H. Funatsu, H. Noma, M. Shimura, Y. Kamei, M. Yoshida, A. Kondo, E. Watanabe, A. Mizota, Aqueous humor levels of cytokines in patients with age-related macular degeneration, Ophthalmologica 241 (2) (2019) 81–89, https://doi.org/10.1159/000490153.

[22] Y. Subhi, M. Krogh Nielsen, C.R. Molbech, A. Oishi, A. Singh, M.H. Nissen, T.L. Sorensen, Plasma markers of chronic low-grade inflammation in polypoidal choroidal vasculopathy and neovascular age-related macular degeneration, Acta Ophthalmol. 97 (1) (2019) 99–106, https://doi.org/10.1111/aos.13886.

[23] Miao YT. Heng, Xiao-xin Li, Inflammatory cytokines in aqueous humor of patients with choroidal neovascularization, Mol. Vis. 18 (2012) 574–580.

[24] S.Y. Kim, S.P. Kambhampati, I.A. Bhutto, D.S. McLeod, G.A. Lutty, R.M. Kannan, Evolution of oxidative stress, inflammation and neovascularization in the choroid and retina in a subretinal lipid induced age-related macular degeneration model, Exp. Eye Res. 203 (2021) 108391, https://doi.org/10.1016/j.exer.2020.108391.

[25] K. Izumi-Nagai, N. Nagai, Y. Ozawa, M. Mihara, Y. Ohsugi, T. Kurihara, T. Koto, S. Satofuka, M. Inoue, K. Tsubota, et al., Interleukin-6 receptor-mediated activation of signal transducer and activator of transcription-3 (STAT3) promotes choroidal neovascularization, Am. J. Pathol. 170 (6) (2007) 2149–2158, https://doi.org/10.2353/ajpath.2007.061018.

[26] T. Tanaka, M. Narazaki, T. Kishimoto, Anti-interleukin-6 receptor antibody, tocilizumab, for the treatment of autoimmune diseases, FEBS Lett. 585 (23) (2011) 3699–3709, https://doi.org/10.1016/j.febslet.2011.03.023.

[27] A. Bograd, P.M. Villiger, M.R. Munk, I. Bolt, C. Tappeiner, Tocilizumab and aflibercept as a treatment option for refractory macular edema after acute retinal necrosis, Ocul. Immunol. Inflamm. 31 (1) (2023) 242–245, https://doi.org/10.1080/09273948.2021.2024580.

[28] M. Leclercq, M. Le Besnerais, V. Langlois, N. Girszyn, Y. Benhamou, C. Ngo, H. Levesque, M. Muraine, J. Gueudry, Tocilizumab for the treatment of birdshot uveitis that failed interferon alpha and anti-tumor necrosis factor-alpha therapy: two cases report and literature review, Clin. Rheumatol. 37 (3) (2018) 849–853, https://doi.org/10.1007/s10067-018-4007-4.

[29] M. Leclercq, A. Andrillon, G. Maalouf, P. Seve, P. Bielefeld, J. Gueudry, T. Sene, T. Moulinet, B. Rouviere, D. Sene, et al., Anti-tumor necrosis factor alpha versus tocilizumab in the treatment of refractory uveitic macular edema: a multicenter study from the French uveitis network, Ophthalmology 129 (5) (2022) 520–529, https://doi.org/10.1016/j.ophtha.2021.11.013.

[30] A.R.Y.S.K. Chung, Effects of subconjunctival tocilizumab versus bevacizumab in treatment of corneal neovascularization in rabbits, Cornea 33 (10) (2014) 1088–1094, https://doi.org/10.1097/ICO.0000000000000220.

[31] T.A. Ferguson, R.S. Apte, Angiogenesis in eye disease: immunity gained or immunity lost? Semin. Immunopathol. 30 (2) (2008) 111–119, https://doi.org/10.1007/s00281-008-0113-8.

[32] D.G. Espinosa-Heidmann, I.J. Suner, E.P. Hernandez, D. Monroy, K.G. Csaky, S.W. Cousins, Macrophage depletion diminishes lesion size and severity in experimental choroidal neovascularization, Invest. Ophthalmol. Vis. Sci. 44 (8) (2003) 3586–3592, https://doi.org/10.1167/iovs.03-0038.

[33] E. Sakurai, A. Anand, B.K. Ambati, N. van Rooijen, J. Ambati, Macrophage depletion inhibits experimental choroidal neovascularization, Investig. Opthalmol. Visual Sci. 44 (8) (2003), https://doi.org/10.1167/iovs.03-0097.

[34] D.M. Mosser, J.P. Edwards, Exploring the full spectrum of macrophage activation, Nat. Rev. Immunol. 8 (12) (2008) 958–969, https://doi.org/10.1038/nri2448.

[35] Z. Liu, X. Mao, Q. Yang, X. Zhang, J. Xu, Q. Ma, Y. Zhou, Q. Da, Y. Cai, A. Sopeyin, et al., Suppression of myeloid PFKFB3-driven glycolysis protects mice from choroidal neovascularization, Br. J. Pharmacol. 179 (22) (2022) 5109–5131, https://doi.org/10.1002/BPH.15925.

[36] Z. Liu, J. Xu, Q. Ma, X. Zhang, Q. Yang, L. Wang, Y. Cao, Z. Xu, A. Tawfik, Y. Sun, et al., Glycolysis links reciprocal activation of myeloid cells and endothelial cells in the retinal angiogenic niche, Sci. Transl. Med. 12 (555) (2020), https://doi.org/10.1126/scitranslmed.aay1371.

[37] S. Droho, A. Rajesh, C.M. Cuda, H. Perlman, J.A. Lavine, CD11c+ macrophages are proangiogenic and necessary for experimental choroidal neovascularization, JCI Insight 8 (7) (2023), https://doi.org/10.1172/jci.insight.168142.

[38] Y. Li, Z. Song, Q. Han, H. Zhao, Z. Pan, Z. Lei, J. Zhang, Targeted inhibition of STAT3 induces immunogenic cell death of hepatocellular carcinoma cells via glycolysis, Mol. Oncol. 16 (15) (2022) 2861–2880, https://doi.org/10.1002/1878-0261.13263.

[39] J. Fu, N. Li, M. He, D. Huang, P. Zhang, STAT3 signaling mediates peritoneal fibrosis by activating hyperglycolysis, Am. J. Transl. Res. 14 (10) (2022) 7552–7565.

[40] M. Hu, R. Bao, M. Lin, X.R. Han, Y.J. Ai, Y. Gao, K.L. Guan, Y. Xiong, H.X. Yuan, ALK fusion promotes metabolic reprogramming of cancer cells by transcriptionally upregulating PFKFB3, Oncogene 41 (40) (2022) 4547–4559, https://doi.org/10.1038/s41388-022-02453-0.

[41] M A, K.K. I U, W.N. Y A, K.K. Y A, N T, Interleukin 6 enhances glycolysis through expression of the glycolytic enzymes hexokinase 2 and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3, J. Nippon Medi. School = Nippon Ika Daigaku zasshi 77 (2) (2022) 97–105, https://doi.org/10.1272/jnms.77.97.
[42] S. Yu, Q. Li, Y. Wang, Y. Cui, Y. Yu, W. Li, F. Liu, T. Liu, Tumor-derived LIF promotes chemoresistance via activating tumor-associated macrophages in gastric cancers, Exp. Cell Res. 406 (1) (2021) 112734, https://doi.org/10.1016/j.yexcr.2021.112734.
[43] F. Hu, X. Sun, G. Li, Q. Wu, Y. Chen, X. Yang, X. Luo, J. Hu, G. Wang, Inhibition of SIRT2 limits tumour angiogenesis via inactivation of the STAT3/VEGFA signalling pathway, Cell Death Dis. 10 (1) (2018) 9, https://doi.org/10.1038/s41419-018-1260-z.