• Basic Research •

Impaired pericyte-Müller glia interaction *via* PDGFRβ suppression aggravates photoreceptor loss in a rodent model of light-induced retinal injury

Wei Xu[1,2,3], *Li-Jin Cui*[1,2,3], *Xiao-Ying Yang*[1], *Xiao-Yuan Cui*[1], *Jian Guo*[1,2,3], *Guo-Xing Xu*[1,2,3]

[1]Department of Ophthalmology, the First Affiliated Hospital of Fujian Medical University, Fuzhou 350001, Fujian Province, China
[2]Department of Ophthalmology, National Regional Medical Center, Binhai Campus of the First Affiliated Hospital of Fujian Medical University, Fuzhou 350212, Fujian Province, China
[3]Fujian Institute of Ophthalmology, Fuzhou 350001, Fujian Province, China

Correspondence to: Guo-Xing Xu. Department of Ophthalmology, the First Affiliated Hospital of Fujian Medical University, Fuzhou 350001, Fujian Provine, China. fjmuxuguoxing@126.com

Received: 2023-05-28 Accepted: 2024-06-21

Abstract

● **AIM:** To investigate the involvement of pericyte-Müller glia interaction in retinal damage repair and assess the influence of suppressing the platelet-derived growth factor receptor β (PDGFRβ) signaling pathway in retinal pericytes on photoreceptor loss and Müller glial response.

● **METHODS:** Sprague-Dawley rats were exposed to intense light to induce retinal injury. Neutralizing antibody against PDGFRβ were deployed to block the signaling pathway in retinal pericytes through intravitreal injection. Retinal histology and Müller glial reaction were assessed following light injury. *In vitro*, normal and PDGFRβ-blocked retinal pericytes were cocultured with Müller cell line (rMC-1) to examine morphological and protein expression changes upon supplementation with light-injured supernatants of homogenized retinas (SHRs).

● **RESULTS:** PDGFRβ blockage 24h prior to intense light exposure resulted in a significant exacerbation of photoreceptor loss. The upregulation of GFAP and p-STAT3, observed after intense light exposure, was significantly inhibited in the PDGFRβ blockage group. Further upregulation of cytokines monocyte chemoattractant protein 1 (MCP-1) and interleukin-1β (IL-1β) was also observed following PDGFRβ inhibition. In the *in vitro* coculture system, the addition of light-injured SHRs induced pericyte deformation and upregulation of proliferating cell nuclear antigen (PCNA) expression, while Müller cells exhibited neuron-like morphology and expressed Nestin. However, PDGFRβ blockage in retinal pericytes abolished these cellular responses to light-induced damage, consistent with the *in vivo* PDGFRβ blockage findings.

● **CONCLUSION:** Pericyte-Müller glia interaction plays a potential role in the endogenous repair process of retinal injury. Impairment of this interaction exacerbates photoreceptor degeneration in light-induced retinal injury.

● **KEYWORDS:** pericyte; Müller glia; light-induced retinal injury; platelet-derived growth factor receptor β; signal pathway

DOI:10.18240/ijo.2024.10.05

Citation: Xu W, Cui LJ, Yang XY, Cui XY, Guo J, Xu GX. Impaired pericyte-Müller glia interaction *via* PDGFRβ suppression aggravates photoreceptor loss in a rodent model of light-induced retinal injury. *Int J Ophthalmol* 2024;17(10):1800-1808

INTRODUCTION

Pericytes are contractile cells that wrap around the endothelial cells to form capillaries, and play significant role in maintaining hemostasis. In addition to their roles in regulating blood flow and preserving blood vessel integrity, pericytes have also been found to play a critical role in tissue repair and regeneration in the retina[1]. This population of cells have been shown to have the ability to differentiate into other cell types, such as smooth muscle cells and even neuron-like cells, and can also produce extracellular matrix proteins that are important for tissue repair[2]. Furthermore, pericytes have been found to release growth factors and cytokines that promote angiogenesis and modulate inflammation[3]. These additional functions of pericytes highlight their versatility and importance in maintaining physiological functions in the retina. Retinal pericyte dysfunction is associated with a range of diseases, such as diabetic retinopathy, retinal vein occlusion, choroidal neovascularization, and even glaucoma[4-5].

With increasing interest in the roles of pericytes in different tissues, there has been growing evidence indicating that pericytes share some features with mesenchymal stem cells, particularly in terms of their expression of cell surface markers and differentiation potential. Recent studies have shown that mesenchymal stem cells can modulate the response of retinal glial cells, including Müller cells, and promote tissue repair in retinal damage[6-7]. Müller cells are the principal glial cells in the retina, and they play an important role in supporting retinal function and maintaining retinal homeostasis. Importantly, Müller cells have been shown to possess a remarkable regenerative capacity and can contribute to the endogenous repair of retinal damage[8-9].

Given the similarities between pericytes and mesenchymal stem cells and the association between mesenchymal stem cells and Müller cells, it is possible that pericytes may also have a role in supporting the function and regeneration of Müller cells in the retina. Several lines of evidence suggested that pericytes and Müller cells may interact with each other and modulate each other's functions in the retina[10-11]. These findings suggest that pericytes may have a broader role in regulating the function and repair of retinal glial cells beyond their known functions in regulating vascular tone and blood flow in the retina. Further studies are needed to elucidate the mechanisms underlying the potential crosstalk between pericytes and Müller cells and to explore the therapeutic potential of pericytes in retinal diseases.

MATERIALS AND METHODS

Ethical Approval All animal experiments in this study were conducted in accordance with ARVO statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Institutional Animal Welfare Ethics Committee (No.NSFC-0365).

Animals Sprague-Dawley (SD) rats, aged 8 to 10wk, were maintained under cyclic light conditions (12h on-off, 80 lx) and provided *ad libitum* access to food and water. Light-induced retinal injury was induced by exposure to cyclic white light conditions (5 klx, color temperature 6500 k to 7000 k) from 8 *p.m*. to 8 *a.m*. with pupil dilation. The experiments were categorized as follows: normal control (Ctrl group), light-induced retinal injury (LI group), light-induced retinal injury receiving platelet-derived growth factor receptor β (PDGFRβ) neutralizing antibody (Neu group) and light-induced retinal injury receiving isotype immunoglobulin G (IgG group). In the Neu group, animals received an intravitreal injection of 2 μL PDGFRβ neutralizing antibody (50 μg/mL, R&D systems, USA) 24h prior to intensive light exposure. The IgG group received an intravitreal isotype of mouse IgG instead (2 μL, 50 μg/mL, R&D systems, Cat. No.MAB1263, USA). After 7d of light exposure, animals were sacrificed for further analysis.

Cell Culture and Treatment Retinas from 4-week-old rats were harvested, digested with 0.2% type I collagenase at 37℃ for 20min, and then suspended by adding fetal bovine serum (FBS; Gibco, USA). The suspension was filtered, collected, and centrifuged before being resuspended in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) supplemented with 20% FBS. The cells were then seeded in a T25 flask and allowed to attach for 72h. The medium was replaced, and cells were passaged when they reached 80%-90% confluence. After cells surface marker identification, the culture medium of retinal pericytes was supplemented with Neu group or mouse IgG group to a final concentration of 50 μg/mL. Pericytes were incubated for 48h and transferred to a coculture system with a Müller glia cell line (rMC-1, Kerafast, Cat. No.ENW001, UK). Supernatants of homogenized retinas (SHRs) were prepared as previously described[12]. Briefly, the retinas after 48-hour light injury were harvested and homogenized in DMEM under sterile conditions. The suspension was centrifuged and the supernatant was collected. Total protein concentration of SHRs were determined. SHRs were added into coculture medium to reach a final concentration of 50 μg/mL. Cells in culture medium without SHRs supplementation were set as Ctrl group, while cells in medium supplemented with SHRs were set as LI group.

Hematoxylin and Eosin Staining The eyeballs were enucleated and fixed in 4% formalin overnight. Subsequently, they were embedded in paraffin, and 5-μm vertical sections were cut using a microtome. The sections were then subjected to hematoxylin and eosin (HE) staining by washing with hematoxylin buffer for 10min at room temperature, followed by a rinse with deionized water and dipping in 1% eosin solution for 15s. After rehydration in a gradient of alcohol solutions, the slices were washed and mounted for microscopy. The histological analysis of retinal tissues was performed using a light microscope, and images of central and peripheral retina were captured for further evaluation.

TUNEL Analysis The eyeballs were embedded in optimal cutting temperature compound at -20℃ overnight, and subsequently, sectioned at 10 μm, fixed in 4% paraformaldehyde for 30min. TUNEL staining was performed using the *in situ* cell death detection kit (Roche, Cat. No.11684795910, USA) following the manufacturer's protocol. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). The sections were examined and photographed using a confocal microscope. For quantitative analysis, TUNEL-positive cell nuclei were counted in central and peripheral retina in each group.

Immunofluorescence Cells or cryosectioned tissues were fixed in 4% paraformaldehyde for 30min and washed in phosphate buffer saline (PBS). After blocking with PBS

containing 10% normal goat serum/0.5% Triton X-100 (for cells) or PBS containing 20% normal goat serum/0.5% Triton X-100 (for tissues) for 1h at room temperature, the following primary antibodies were applied overnight at 4°C: mouse monoclonal anti-PDGFRβ, neural/glial antigen 2 (NG2), CD31, proliferating cell nuclear antigen (PCNA), Nestin (all 1:200, Santa Cruz, Cat. No. sc-374573, sc-33666, sc-376764, sc-25280, sc-33677, USA), rabbit polyclonal anti-glutamine synthetase (GS) (1:1000, Abcam, Cat. No. ab228590, USA). Secondary antibodies conjugated to fluorescein isothiocyanate (FITC) or tetramethylrhodamine isothiocyanate (TRITC; Sigma, St. Louis, MO, USA) were diluted 1:200 and incubated for 2h at room temperature. Nuclei were counterstained with DAPI, and confocal microscopy was used to capture images for further analysis.

Western Blot Analysis The protein was extract from retinas and the concentration of each sample was determined. The samples were denaturated and equally loaded. Proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) using 8%-10% gels and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with blocking buffer (Beyotime, China) and incubated with primary antibodies at 4ºC overnight. The following primary antibodies were used: Mouse monoclonal anti-GFAP, PCNA, p-STAT3 (all 1:500, Santa Cruz, Cat. No.sc-33673, sc-25280, sc-8059), mouse monoclonal anti-Rhodopsin, β-actin (all 1:1000, Abcam, Cat. No.ab98887, ab6276), rabbit polyclonal anti-MCP-1 (1:1000, Abcam, Cat. No.ab7202) and rabbit monoclonal anti-interleukin-1β (IL-1β; 1:1000, Abcam, Cat. No.ab283818). After washing, the primary antibodies were detected by incubating the membranes with horse radish peroxidase (HRP)-conjugated secondary antibodies for 2h. Protein bands were detected using Beyo ECL Plus Kit (Beyotime, China).

Statistical Analysis The normally distributed data were presented as mean±standard deviation (SD) and analyzed using one-way ANOVA for comparison among groups in GraphPad Prism 9 (GraphPad Software, USA). Statistical significance was set at $P<0.05$.

RESULTS

Histological Changes in Light-Induced Retinal Injury with PDGFRβ Suppression Histological analysis revealed significant damage to the outer layer of the retina exposure to intense light. In Ctrl group HE staining showed a clear and organized outer nuclear layer (ONL) and intact outer segment (OS). However, periodic exposure to intense light for 7d caused significant ONL structure loss and OS disorder (LI group; Figure 1A). The samples exhibited detachment of the retina from pigment epithelial layer and choroidal tissue. Furthermore, both central and peripheral regions of the retina showed a reduction in the number of nuclei in the ONL and a decrease in ONL thickness (LI group versus Ctrl group, $P<0.05$; Figure 1B). Prior to 24h of light exposure, Neu group significantly reduced ONL nucleus count compared to LI group and IgG group (Neu group versus LI group and IgG group, $P<0.05$).

Retinal Cells Apoptosis TUNEL assay was employed to investigate the effect of intense light exposure on retinal cell death. Little apoptotic activity was observed in Ctrl group, while 7d of intense light exposure resulted in the appearance of TUNEL-positive cells primarily located in the ONL accompanying with a decrease in ONL thickness (LI group; Figure 2A). PDGFRβ signal pathway inhibition by neutralizing antibodies intravitreal injection significantly increased apoptotic cells in the central region of the retina (Neu group versus LI group, $P<0.05$; Figure 2B), while the IgG isotype injection group showed similar results with light injury group. Although an increase in apoptotic cells was also observed in the peripheral retina, statistical significance was not identified (Neu group versus LI group, $P>0.05$). These findings suggest that PDGFRβ pathway inhibition could exacerbate intense light exposure-induced photoreceptor apoptosis.

Endogenous Response in Retinas To investigate the endogenous repair of retina in response to intense light exposure, we employed Western blot assay to detect protein changes in the retinas after 7d of exposure. Our results showed that retinal light damage induced a glial cell response and activated a cell survival signal pathway, upregulating the expression of GFAP and p-STAT3 proteins (Ctrl group versus LI group, $P<0.05$; Figure 3A and 3B). Importantly, inhibition of the PDGFRβ pathway weakened the glial cell response and downregulated GFAP and p-STAT3 expression (Neu group versus LI group, $P<0.05$; Figure 3B and 3C). Additionally, rhodopsin expression was decreased in the group exposed to intense light, and this decrease was further observed in the group pretreated with PDGFRβ neutralized antibodies (Neu group versus LI group, $P<0.05$; Figure 3F). Furthermore, the expression of PCNA was upregulated following retinal light damage (Ctrl group versus LI group, $P<0.05$; Figure 3E), indicating involvement of endogenous repair mechanisms. However, PDGFRβ suppression led to a downregulation of this antigen expression (Neu group versus LI group, $P<0.05$), suggesting a decrease ability in retinal endogenous repair. Our results also showed that light-induced retinal injury triggered an increase in the expression of proinflammatory cytokines, IL-1β and MCP-1, confirming the presence of an inflammatory response in the retina. Interestingly, inhibiting the PDGFRβ pathway resulted in a significant upregulation of these

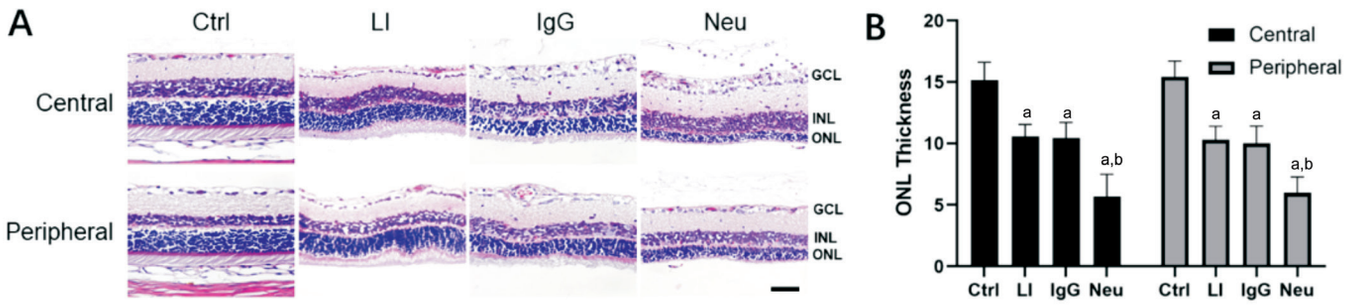

Figure 1 Histological changes in light-induced retinal injury among groups A: The control group exhibited a clear and organized ONL and intact outer segments, as evidenced by hematoxylin and eosin staining. However, exposure to intense light for 7d resulted in significant damage to the outer layer of the retina, with detachment from the pigment epithelial layer and choroidal tissue observed. B: Both central and peripheral regions of the retina demonstrated a reduction in ONL thickness. Pre-treatment with neutralizing antibodies against PDGFRβ *via* intravitreal injection prior to 24h of light exposure resulted in a significant reduction in ONL thickness compared to both LI group and IgG group. GCL: Ganglion cell layer; INL: Inner nuclear layer; ONL: Outer nuclear layer; Ctrl: Control group; LI: Light injury group; IgG: IgG isotype injection group; Neu: PDGFRβ neutralizing antibody injection group; PDGFRβ: Platelet-derived growth factor receptor β. [a]*P*<0.05 compared to Ctrl, [b]*P*<0.05 compared to LI; *n*=7; bar=50 μm.

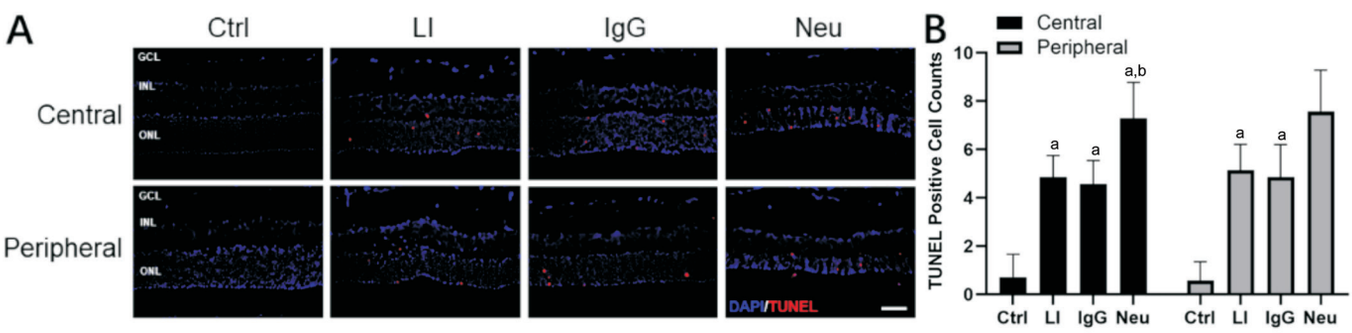

Figure 2 Retinal cell death after intense light exposure A: In the normal retina, few TUNEL-positive cells were detected, whereas intense light exposure resulted in apoptosis primarily located in the ONL, accompanied by a decrease in ONL thickness. B: Neu group significantly increased the number of apoptotic cells in the central region of the retina. However, the IgG group displayed no significant change in contrast to the light injury group. GCL: Ganglion cell layer; INL: Inner nuclear layer; ONL: Outer nuclear layer; Ctrl: Control group; LI: Light injury group; IgG: IgG isotype injection group; Neu: PDGFRβ neutralizing antibody injection group; PDGFRβ: Platelet-derived growth factor receptor β. [a]*P*<0.05 compared to Ctrl, [b]*P*<0.05 compared to LI; *n*=7; bar=50 μm.

inflammatory mediators (Neu group versus LI group, *P*<0.05; Figure 3H and 3I), indicating that suppression of PDGFRβ exacerbates the inflammatory response within the retina.

Interactions Between Pericytes and Müller Glia *In vitro* cultured retinal pericytes were identified by immunofluorescence staining to be positive for surface markers NG2 and PDGFRβ, while negative for endothelial cell marker CD31 (Figure 4). To study the interplay between retinal pericytes and Müller glia through the inhibition of the PDGFRβ signaling pathway in retinal pericytes, we transferred retinal pericytes and Müller glia into an *in vitro* coculture system. We found that retinal pericytes undergo morphological changes and exhibit increased PCNA expression after 48h of light-injured SHRs stimulation (LI group; Figure 5). However, when retinal pericytes were pretreated with PDGFRβ neutralizing antibodies for 48h prior to SHRs stimulation (Neu group), the morphological changes disappeared and the cells maintained a spindle-like shape, with PCNA expression similar to the control group. In Müller glia, we observed positive Nestin expression and elongated cell bodies with ramified protrusions connecting neighboring cells, resembling the appearance of neurons, after 48h of SHRs stimulation. However, in the coculture system with retinal pericytes in which PDGFRβ signaling pathway was inhibited, the neuronal morphological changes in Müller glial cells disappeared and the number of Nestin-positive cells decreased, indicating that the integrity of retinal pericyte function is essential for Müller glial cells to respond to light-induced injury. A mutual interaction between retinal pericytes and Müller glial cells is implicated for endogenous repair of the retina.

In vivo study, we also observed scattered expression of Nestin in Müller glial cells within LI group and IgG group. The immunofluorescence staining revealed the presence of positive staining for GS and Nestin in the LI group and IgG group, respectively, with colocalization of these markers (Figure 6). Remarkably, positively stained cells exhibited

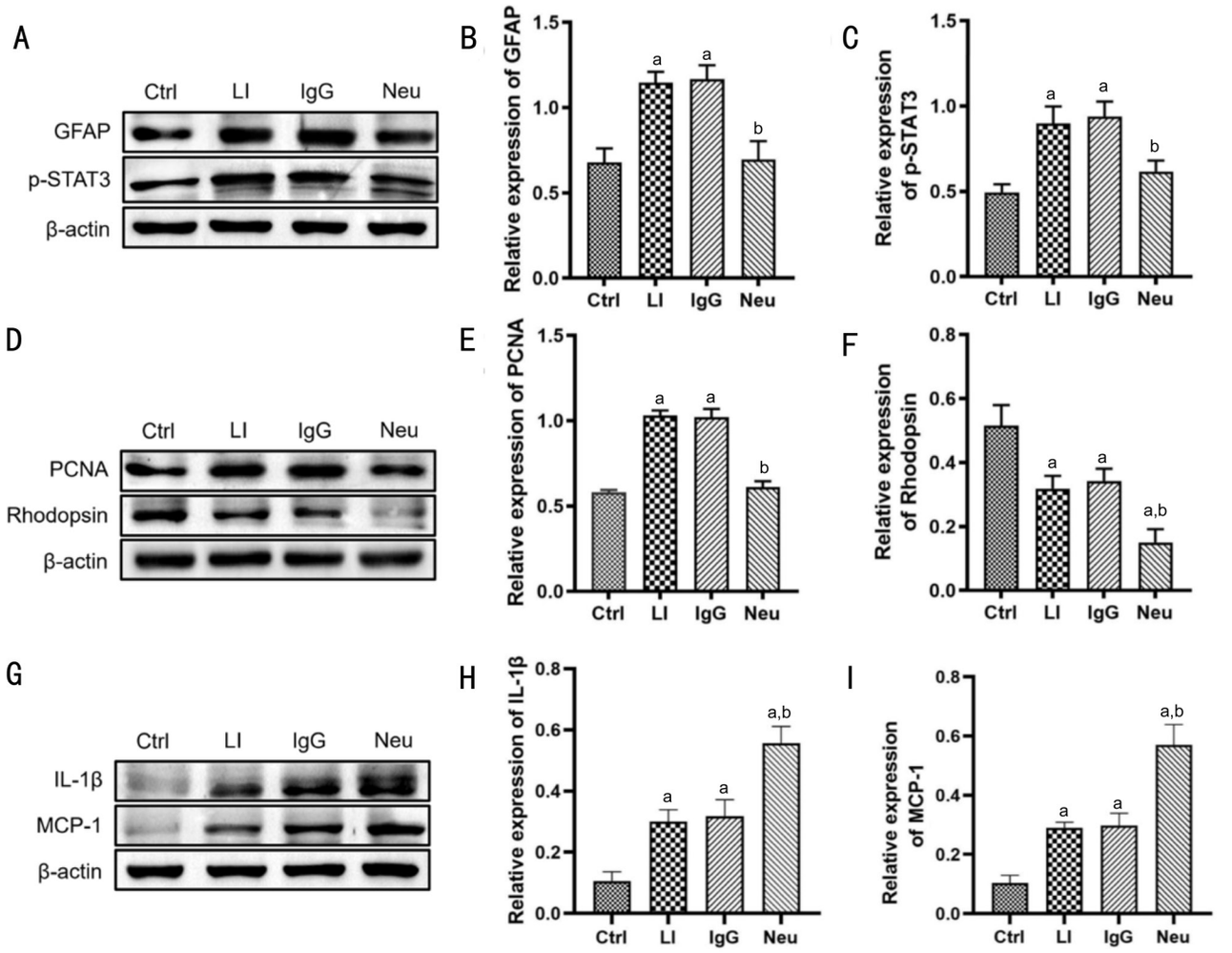

Figure 3 Endogenous response in the retina after intense light exposure Exposure to intense light resulted in the upregulation of GFAP and p-STAT3 in the retina (A). However, the inhibition of the PDGFRβ pathway led to a significant downregulation of GFAP and p-STAT3 expression (B, C). Rhodopsin expression decreased after exposure to intense light (D), and a further decrease was observed in the group that was pretreated with PDGFRβ neutralizing antibody injection (F). The upregulation of PCNA following retinal light damage was suppressed in the group with PDGFRβ neutralizing antibody (E). Moreover, light-induced retinal injury triggered upregulation of IL-1β and MCP-1 (G), and the suppression of PDGFRβ exacerbated overexpression of these cytokines within the retina (H, I). Ctrl: Control group; LI: Light injury group; IgG: IgG isotype injection group; Neu: PDGFRβ neutralizing antibody injection group; PDGFRβ: Platelet-derived growth factor receptor β; PCNA: Proliferating cell nuclear antigen; IL: Interleukin. [a]P<0.05 compared to Ctrl, [b]P<0.05 compared to LI; n=5.

long processes penetrating the outer nuclear layer, signs of neuronal transdifferentiation in Müller cells. Following intravitreal injection of PDGFRβ neutralizing antibodies (Neu group), neuronal transdifferentiation in Müller cells was not observed. Therefore, in response to light-induced damage, the retina activates an endogenous repair mechanism partially by Müller glia transdifferentiation, and inhibition of the PDGFRβ pathway results in a loss of this reparative function.

DISCUSSION

Pericytes are one of the key components of the neurovascular unit in maintaining the stability of neural network. The intimate anatomical and functional interactions between pericytes and other neurovascular unit components play roles in the progression of multiple neural disorders[13]. In this study, we investigated the role of pericytes-Müller glia interaction in the endogenous repair of light-induced retinal injury. Our study revealed that inhibition of PDGFRβ signaling in pericytes altered their response to retinal light damage. Specifically, pericytes showed reduced cell deformation ability and downregulation of PCNA expression, a marker of cell proliferation, while Müller glia exhibited reduced ability to participate in light injury repair after pericyte dysfunction. Weakened interaction between pericytes and Müller glial cells compromised the endogenous repair of retinal damage from intense light exposure. Müller glia expressed neural progenitor marker upon light damage stimulation, but this phenomenon was reversed under PDGFRβ signaling inhibition in pericytes, suggesting that pericytes may play a role in promoting Müller glia transdifferentiation through PDGFRβ signaling-related pathways.

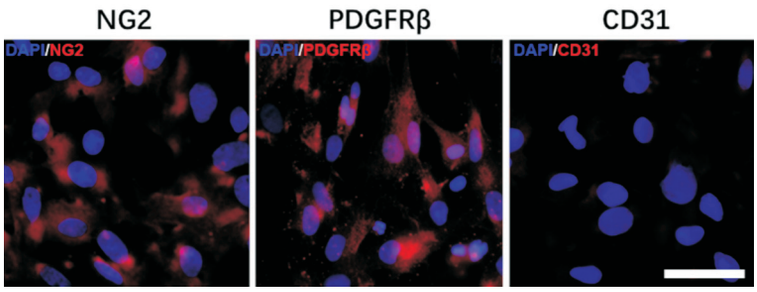

Figure 4 Identification of retinal pericyte Immunofluorescence showed *in vitro* cultured pericytes were positive for NG2, PDGFRβ and negative for endothelium marker CD31. Bar=50 μm. PDGFRβ: Platelet-derived growth factor receptor β; NG2: Neural/glial antigen 2.

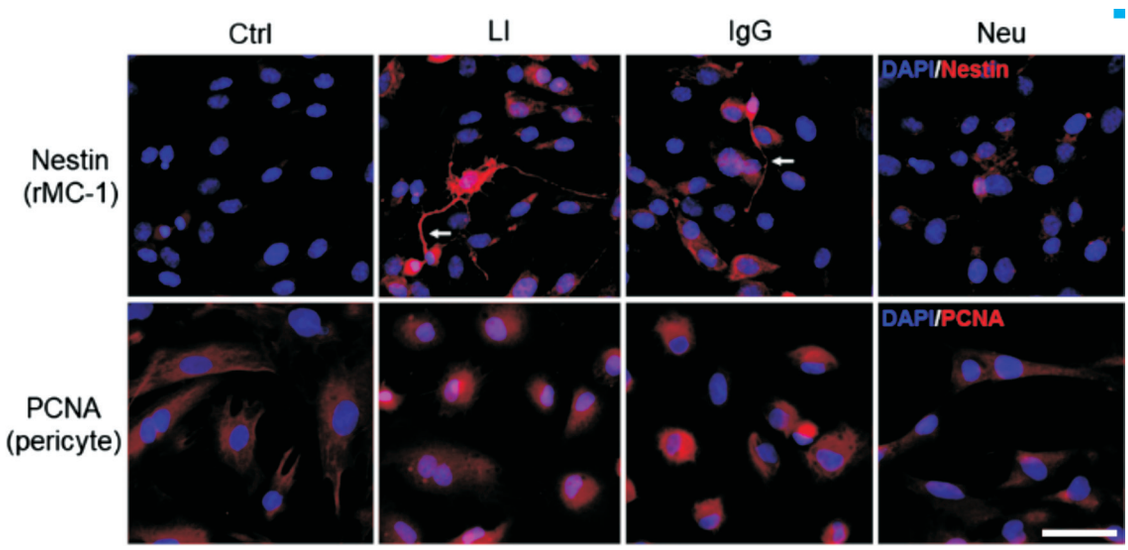

Figure 5 PDGFRβ blockage in pericyte compromised pericyte-Müller glia interaction When cocultured with retinal pericytes in control medium, the Müller glia cell line rMC-1 showed dark staining for Nestin. However, after 48h of SHRs stimulation, the cells exhibited positive Nestin expression, elongated cell bodies, and ramified protrusions connecting neighboring cells (arrows). PDGFRβ neutralizing antibody block in pericytes reversed the morphological changes in Müller glia and reduced the number of Nestin-positive cells. Following light-induced SHRs stimulation, retinal pericytes displayed a round morphology and increased PCNA expression. When pericytes were pretreated with PDGFRβ neutralizing antibody prior to SHRs stimulation, the morphological changes disappeared, and the cells maintained a spindle-like shape, with PCNA expression similar to the control group. Ctrl: Control group; LI: Light injury group; IgG: IgG isotype injection group; Neu: PDGFRβ neutralizing antibody injection group; PDGFRβ: Platelet-derived growth factor receptor β; PCNA: Proliferating cell nuclear antigen; SHRs: Supernatants of homogenized ratinal; Bar=50 μm.

In the retina, the composition of pericytes is exceptionally abundant. The ratio of pericytes to endothelial cells even reaches up to 1/1, whereas in other tissues with rich microvasculature, such as the kidney and brain, the ratio is relatively lower, at approximately 2/5 and 1/5, respectively[14]. Therefore, the presence of abundant pericytes in the retina is critical in maintaining retinal hemostasis. Pericytes promote the survival of endothelial cells in response to vessel insults. They have been shown to possess the ability to migrate and recruit to designated sites through the action of PDGF-B during angiogenesis. Pericytes are also an important source of vascular endothelial growth factor (VEGF), Ang-1 and TGF-β to exert a paracrine effect[15-17]. Depending on their location in the microvasculature, pericytes in the retina have different functions. Pre-capillary pericytes participate in regulating blood flow. Pericytes in the capillary maintain the blood-retinal barrier, while those in the post-capillary venule modulate inflammation response in tissue injury[18-19]. Pericytes may regulate retinal blood flow under stress to adjust energy cascade and facilitate clearance of metabolic products, thereby protecting retina from excessive light exposure. We observed deformation of pericytes in response to light-induced retinal injury. However, the deficiency of PDGFRβ signal pathway in pericytes reduces this effect and exacerbates photoreceptor loss. Pericytes can undergo corresponding morphological and functional changes in response to different situation, even expressing different surface markers. The absence of an exclusive marker for pericytes is also one of the difficulties in pericyte identification, and multiple markers are often recommended. In this study, retinal pericytes were identified

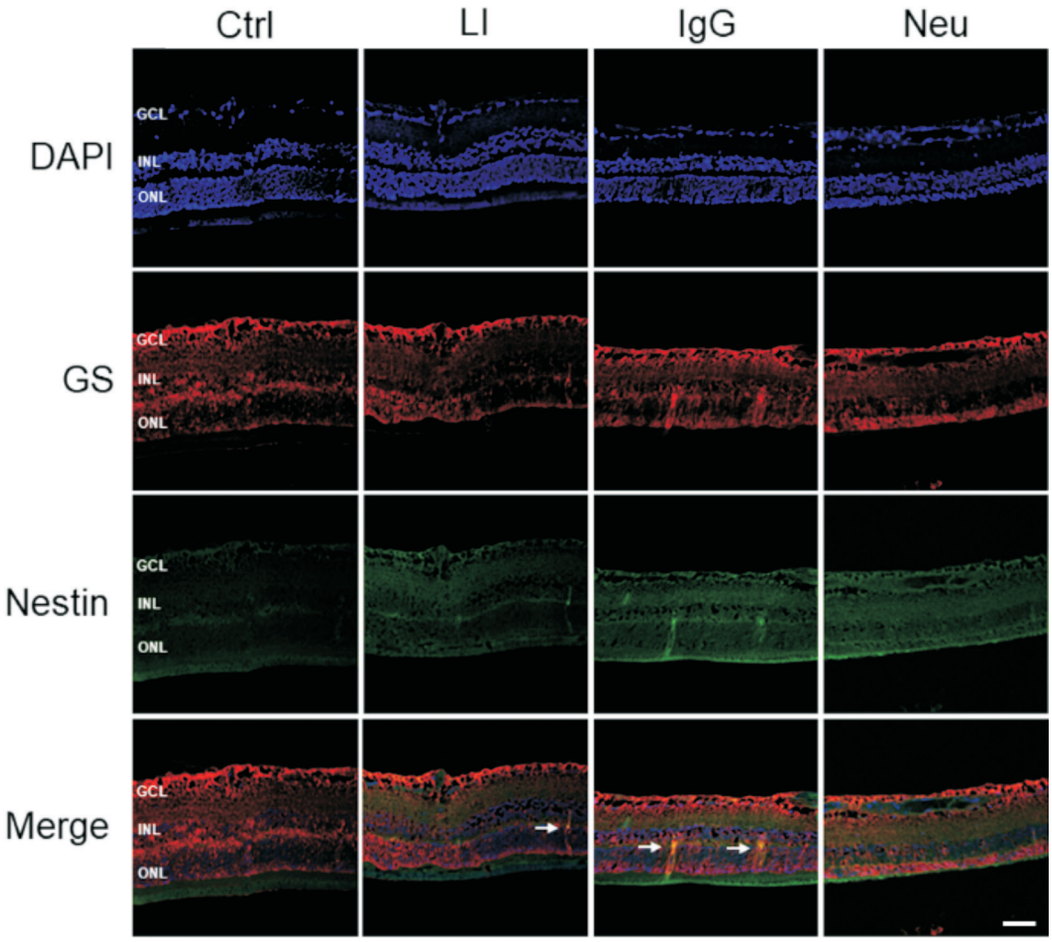

Figure 6 PDGFRβ blockage *in vivo* inhibited Müller glia response Nestin expression was not detectable in the normal retina. However, sporadic Nestin-positive expression was observed in the light-injured retina group and IgG isotype injection group, mainly localized in the outer plexiform layer of the retina. The Nestin-positive cells in these groups exhibited elongated processes that penetrated the outer layer (arrows), but this Nestin-positive expression was absent in the PDGFRβ neutralizing antibody injection group. GCL: Ganglion cell layer; INL: Inner nuclear layer; ONL: Outer nuclear layer; Ctrl: Control group; LI: Light injury group; IgG: IgG isotype injection group; Neu: PDGFRβ neutralizing antibody injection group; PDGFRβ: Platelet-derived growth factor receptor β; GS: Glutamine synthetase. Bar=50 μm.

both NG2 and PDGFRβ positive to ensure homogeneity of the cell population and minimal interference by glial cell or vascular smooth muscle cell.

Müller glia is a type of cell that spans all retinal layers and contacts with neighboring neurons. They also play an important role in maintaining retinal homeostasis and contribute to the structure and function of the retina, and act as barriers and conduits for the transfer of various molecules between different retinal cells and compartments[20-21]. These cells can support neurons by releasing trophic factors, recycling neurotransmitters, and controlling the ionic balance in the extracellular space. In addition, Müller glia are known to participate in retinal regeneration and repair following injury or disease. They can differentiate into retinal progenitor cells and undergo proliferation and migration to replace damaged or lost retinal cells[22]. We found that Müller glia developed neuronal morphology and expressed Nestin *in vitro* when receiving stimulation from light-induced retinal injury, while this appearance disappeared after pericytes dysfunction. A similar result was observed *in vivo*, where colocalization of Nestin and GS were unable to be detected in the retinas with PDGFRβ inhibition. To further detect cell survival signal pathway, our results showed that increased phosphorylation of STAT3 in light-induced retinal injury was significantly inhibited when pericyte dysfunction occurred. Since Müller glia have been reported to involve in cell survival in multiple retinal disease models through STAT3 pathway[23], change of p-STAT3 expression in our study might be a downstream event from weakened interactions between pericytes and Müller glia. However, further investigation is required to understand the signal transduction. Another finding of our study was that retinal inflammation markers, MCP-1 and IL-1, were significantly upregulated following the inhibition of the PDGFR signaling pathway. As pericytes play a critical role in immunomodulation, their dysfunction resulting from PDGFR signaling pathway inhibition may compromise the ability to

suppress inflammation by light-induced retinal injury. This, in turn, may exacerbate the cell death of retina when exposure to intense light.

In this study, we employed PDGFRβ neutralizing antibodies to induce pericyte dysfunction. Our findings indicated that this approach effectively suppressed pericyte function. The use of PDGFRβ neutralizing antibodies against pericytes has also been shown to efficiently inhibit choroidal neovascularization in a rodent animal model, with a similar inhibitory effect to the method of PDGFRβ knockdown[24]. However, it's worth noting that inhibiting PDGFRβ in the retina may also partially impair the function of Müller glia, as PDGFRβ is expressed in these cells. Therefore, while this approach can be useful for inhibiting pericyte function *in vivo*, it has limitations that highlight the need for further research into alternative signaling pathways involved in pericyte function. The interaction between pericytes and Müller glia in response to retinal injury was examined through morphological changes and immunofluorescence. To gain a comprehensive understanding, further investigations into the transcriptomic and functional changes of these cells are necessary for addressing the current limitations.

In summary, light-induced retinal injury involves in a complex process of photoreceptor cell death, cytokines release, and endogenous repair mechanisms. The interactions between retinal pericytes and Müller cells play significant roles in this process. Our study reveals that inhibition of the PDGFRβ pathway results in compromised crosstalk between pericytes and Müller glia, which further exacerbates the loss of photoreceptors in light-injured retina.

ACKNOWLEDGEMENTS

Foundation: Supported by National Natural Science Foundation of China (No.81900862).

Conflicts of Interest: Xu W, None; **Cui LJ,** None; **Yang XY,** None; **Cui XY,** None; **Guo J,** None; **Xu GX,** None.

REFERENCES

1 Trost A, Bruckner D, Rivera FJ, Reitsamer HA. Pericytes in the retina. *Adv Exp Med Biol* 2019;1122:1-26.

2 Fujii-Tezuka R, Ishige-Wada M, Nagoshi N, Okano H, Mugishima H, Takahashi S, Morioka I, Matsumoto T. Umbilical artery tissue contains p75 neurotrophin receptor-positive pericyte-like cells that possess neurosphere formation capacity and neurogenic differentiation potential. *Regen Ther* 2021;16:1-11.

3 Ogura S, Kurata K, Hattori Y, *et al*. Sustained inflammation after pericyte depletion induces irreversible blood-retina barrier breakdown. *JCI Insight* 2017;2(3):e90905.

4 Alarcon-Martinez L, Shiga Y, Villafranca-Baughman D, Belforte N, Quintero H, Dotigny F, Cueva Vargas JL, Di Polo A. Pericyte dysfunction and loss of interpericyte tunneling nanotubes promote neurovascular deficits in glaucoma. *Proc Natl Acad Sci U S A* 2022;119(7):e2110329119.

5 Xia HQ, Yang JR, Zhang KX, Dong RL, Yuan H, Wang YC, Zhou H, Li XM. Molecules related to diabetic retinopathy in the vitreous and involved pathways. *Int J Ophthalmol* 2022;15(7):1180-1189.

6 Jha KA, Gentry J, Del Mar NA, Reiner A, Sohl N, Gangaraju R. Adipose tissue-derived mesenchymal stem cell concentrated conditioned medium alters the expression pattern of glutamate regulatory proteins and aquaporin-4 in the retina after mild traumatic brain injury. *J Neurotrauma* 2021;38(12):1702-1716.

7 Zhang J, Li PD, Zhao GF, He SQ, Xu D, Jiang WJ, Peng Q, Li ZH, Xie ZJ, Zhang H, Xu Y, Qi L. Mesenchymal stem cell-derived extracellular vesicles protect retina in a mouse model of retinitis pigmentosa by anti-inflammation through miR-146a-Nr4a3 axis. *Stem Cell Res Ther* 2022;13(1):394.

8 Todd L, Volkov LI, Zelinka C, Squires N, Fischer AJ. Heparin-binding EGF-like growth factor (HB-EGF) stimulates the proliferation of Müller glia-derived progenitor cells in avian and murine retinas. *Mol Cell Neurosci* 2015;69:54-64.

9 Liu Y, Wang CG, Su GF. Cellular signaling in Müller glia: progenitor cells for regenerative and neuroprotective responses in pharmacological models of retinal degeneration. *J Ophthalmol* 2019;2019:5743109.

10 Hu J, Dziumbla S, Lin JH, *et al*. Inhibition of soluble epoxide hydrolase prevents diabetic retinopathy. *Nature* 2017;552(7684):248-252.

11 Kugler EC, Greenwood J, MacDonald RB. The "neuro-glial-vascular" unit: the role of glia in neurovascular unit formation and dysfunction. *Front Cell Dev Biol* 2021;9:732820.

12 Xu W, Wang XT, Xu GX, Guo J. Basic fibroblast growth factor expression is implicated in mesenchymal stem cells response to light-induced retinal injury. *Cell Mol Neurobiol* 2013;33(8):1171-1179.

13 Cai W, Liu H, Zhao JY, Chen LY, Chen J, Lu ZQ, Hu XM. Pericytes in brain injury and repair after ischemic stroke. *Transl Stroke Res* 2017;8(2):107-121.

14 Rudziak P, Ellis CG, Kowalewska PM. Role and molecular mechanisms of pericytes in regulation of leukocyte diapedesis in inflamed tissues. *Mediators Inflamm* 2019;2019:4123605.

15 Dubrac A, Künzel SE, Künzel SH, Li JY, Chandran RR, Martin K, Greif DM, Adams RH, Eichmann A. NCK-dependent pericyte migration promotes pathological neovascularization in ischemic retinopathy. *Nat Commun* 2018;9(1):3463.

16 Khan M, Aziz AA, Shafi NA, Abbas T, Khanani AM. Targeting angiopoietin in retinal vascular diseases: a literature review and summary of clinical trials involving faricimab. *Cells* 2020;9(8):1869.

17 Corliss BA, Ray HC, Doty RW, *et al*. Pericyte bridges in homeostasis and hyperglycemia. *Diabetes* 2020;69(7):1503-1517.

18 Attwell D, Mishra A, Hall CN, O'Farrell FM, Dalkara T. What is a pericyte? *J Cereb Blood Flow Metab* 2016;36(2):451-455.

19 Li M, Huang JF, Li Y, Shen J, Yang LJ, Chen Q, Zhang QP, Yi XN. Dynamic changes of inducible nitric oxide synthase expression in rat's retina and its role on blood-retinal barrier injury after acute high intraocular pressure. *Int J Ophthalmol* 2022;15(7):1053-1061.

20 Eastlake K, Lamb WDB, Luis J, Khaw PT, Jayaram H, Limb GA. Prospects for the application of Müller glia and their derivatives in retinal regenerative therapies. *Prog Retin Eye Res* 2021;85:100970.

21 Zhao YM, Sun RS, Duan F, Wang FY, Li YJ, Qian XB, Zeng JT, Yang Y, Lin XF. Intravitreal slow-release dexamethasone alleviates traumatic proliferative vitreoretinopathy by inhibiting persistent inflammation and Müller cell gliosis in rabbits. *Int J Ophthalmol* 2023;16(1):22-32.

22 Goldman D. Müller glial cell reprogramming and retina regeneration. *Nat Rev Neurosci* 2014;15(7):431-442.

23 Eichler W, Savković-Cvijić H, Bürger S, Beck M, Schmidt M, Wiedemann P, Reichenbach A, Unterlauft JD. Müller cell-derived PEDF mediates neuroprotection via STAT3 activation. *Cell Physiol Biochem* 2017;44(4):1411-1424.

24 Strittmatter K, Pomeroy H, Marneros AG. Targeting platelet-derived growth factor receptor β(+) scaffold formation inhibits choroidal neovascularization. *Am J Pathol* 2016;186(7):1890-1899.

CORRIGENDUM

Superior rectus/levator complex in acquired anophthalmic socket repaired with spheric implant—a computed tomography scan and topographic study

Shaikha Al Eid, Hamad M AlSulaiman, Sahar M Elkhamary, Osama Al Sheikh, Alicia Ferrero-Galindo, Rajiv Kandekhar, Silvana A Schellini

(*Int J Ophthalmol* 2024;17(8):1483-1488. DOI:10.18240/ijo.2024.08.14)

The authors would like to make the following change to the above article:

The name of the author Alicia Ferrero-Galindo should be modified as “Alicia Galindo-Ferreiro”.

The authors apologize for any inconvenience caused by this error.