Molecular Vision 2024; 30:410-420 <http://www.molvis.org/molvis/v30/410>
Received 27 March 2024 | Accepted 2 December 2024 | Published 4 December 2024

Peroxisome proliferator-activated receptor-α affects corneal epithelial wound healing

Yang Wu,[1] Jinghua Bu,[2] Rongrong Zhang,[2] Le Sun,[2] Yuanzhi Yuan,[1] Wei Li[2,3]

(The first two authors contributed equally to this work)

[1]Zhongshan Hospital (Xiamen), Fudan University, Xiamen, Fujian, China; [2]Department of Ophthalmology, Xiang'an Hospital of Xiamen University; Eye Institute of Xiamen University; Fujian Provincial Key Laboratory of Ophthalmology and Visual Science; Fujian Engineering and Research Center of Eye Regenerative Medicine; School of Medicine, Xiamen University. Xiamen, Fujian, China; [3]Xiamen University affiliated Xiamen Eye Center, Xiamen, Fujian, China

Purpose: This study aimed to determine the role of peroxisome proliferator-activated receptor α (PPARα) on corneal epithelial wound healing.
Methods: Ten-week-old PPARα knockout (*PPARα*$^{-/-}$) mice and wild-type (WT) C57BL/6 mice and ex vivo cultured human corneal epithelial cells were used to investigate the function of PPARα on corneal epithelial wound healing. A two-millimeter diameter of the mice's central corneal epithelium was removed to induce corneal epithelial injury. The expression of PPARα during corneal epithelial wound healing was analyzed using immunofluorescent staining and quantitative RT–PCR. Histological and immunostaining techniques were used to evaluate corneal morphology, cell proliferation, and inflammatory response in WT and *PPARα*$^{-/-}$ mice. PPARα agonist fenofibrate was used to determine its effect on corneal epithelial wound healing.
Results: PPARα expression was found to significantly increase during corneal epithelial repair. *PPARα*$^{-/-}$ mice exhibited delayed corneal epithelial wound healing compared to WT mice. *PPARα*$^{-/-}$ mice displayed altered proliferative responses and distinct patterns of inflammatory infiltrates. Administration of fenofibrate to WT mice resulted in accelerated corneal epithelial repair and increased PPARα expression and cell proliferation. In vitro studies using human corneal epithelial cells further supported the impact of fenofibrate on promoting corneal epithelial cell wound healing.
Conclusions: PPARα is a regulator of corneal epithelial wound healing, and its absence leads to delayed repair processes in the corneal epithelium.

The corneal epithelium covers the outermost surface of the cornea tissue and plays an important role in protecting the eye from, physical and chemical damage and microbial infections from the external environment. Many different factors can cause wounding of the corneal epithelia, and it has the property of healing in a relatively short time.

Peroxisome proliferator-activated receptor (PPAR), a nuclear receptor superfamily, is a class of structurally highly conserved, ligand-dependent transcriptional regulators, including PPARα, PPARβ/δ, and PPARγ. These are widely distributed in various tissues and organs and are closely related to the functions of lipid metabolism and inflammation regulation [1]. PPAR subtypes have similar structures and functional patterns but differ in tissue distribution, ligand specificity, and functional focus [2,3].

Correspondence to: Wei Li, Xiang'an Hospital of Xiamen University, Eye Institute of Xiamen University, School of Medicine, Xiamen University. Xiamen, Fujian, China;The 4th Floor, 4221-122, South Xiang'an Rd, Xiamen, Fujian, 361102, China; email: wei1018@xmu.edu.cn

PPARα is the first identified PPAR family member [4], and it is highly expressed in the liver, heart, kidney, brown adipose tissue, muscle, and intestines [5,6]. It behaves as an important modulator of T-cell functions and autoimmune pathologies [7,8], plays a major regulatory function in the genes involved in energy metabolism, lipid homeostasis, and inflammation control [9], and is involved in skin development and wound repair [10,11]. More specifically, PPARα is mainly involved in the early inflammation phase of skin wound healing [11].

Zhou et al. found that fenofibrate exerts anti-neovascularization and anti-inflammatory effects on the cornea by regulating the key enzymes of lipid metabolism and ameliorating the lipid peroxidation damage of the cornea through the PPARα signaling pathway [12]. Liang et al. identified PPARα as being a crucial regulator of mitochondrial function in the corneal epithelium, and the downregulation of diabetes-induced PPARα contributes to impaired wound healing in the diabetic cornea [13].

PPARα downregulation plays a pathogenic role in deficiencies in wound healing in the diabetic cornea [13].

However, temporal changes in PPARα during corneal epithelial injuries in the non-diabetic cornea and its specific impacts on corneal epithelial cells and the surrounding inflammatory environment across the wound healing process remain unclear. Investigating this question is thus the main objective of this study. The results of this study may provide valuable insights into the underlying mechanisms of corneal wound healing and identify potential therapeutic targets to promote more efficient healing processes.

METHODS

Animals: PPARα knockout (*PPARα*$^{-/-}$) mice and wild-type (WT) C57BL/6J mice aged 10 weeks were used in the experiments. Adult male C57BL/6J mice were purchased from Shanghai SLAC Laboratory Animal Company Ltd. (Shanghai, China). *PPARα*$^{-/-}$ mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All studies were performed in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. This study also received approval from the Animal Ethical Committee of Xiamen University. Animals were provided free access to standard rodent chow and water and were housed in a standard pathogen-free environment that was maintained at a constant temperature of 25 °C±1 °C, with a relative humidity of 60%±10%. They were subjected to alternating 12 h light–dark cycles (from 8:00 AM to 8:00 PM) to emulate natural lighting conditions.

Animal treatment: Experimental mice were intraperitoneally narcotized with ketamine (80 mg/kg) and xylazine (10 mg/kg) and then locally anesthetized by using a topical administration of 0.4% oxybuprocaine hydrochloride. The central corneal epithelia were then demarcated with a 2 mm trephine and removed using an Algerbrush II rotating burr (Alger Equipment Co., Inc., Lago Vista, TX) under the operating microscope. A volume of 1 µl of 1% liquid sodium fluorescein (Jingmingxin Co., Ltd., Tianjin, China) was instilled into the mice's conjunctival sac. Corneal epithelial fluorescein staining was documented 90 s after application using a slit-lamp microscope equipped with a cobalt blue filter at 12, 24, 36, and 48 h post-injury. The area of fluorescein sodium staining was then recorded using our previous method [14]. All images were captured using the same camera and settings throughout the study, and this was done under the guidance of a single ophthalmologist. The mice were subjected to humane euthanasia by administering a pentobarbital overdose (100 mg/kg) immediately after surgical ablation, as well as at 12, 24, 36, and 48 h post-injury. After euthanasia, corneal epithelia were harvested for RNA extraction, or eyeballs were collected for histology and immunostaining.

Fenofibrate treatment: WT mice were subjected to fenofibrate eye drops four times a day throughout the corneal repair process. As shown in our previous report [15], the eye drops consisted of either 200 µM fenofibrate dissolved in dimethyl sulfoxide (DMSO) and subsequently diluted with saline or a corresponding vehicle solution of saline containing an equivalent volume of DMSO (same volume as 200 µM fenofibrate, 0.1% DMSO) [15]. At 24 h post injury, the animals were euthanized by administering a pentobarbital overdose (100 mg/kg). Their eyes were enucleated, and either the corneal epithelia were dissected for total RNA extraction or their eyeballs were collected for immunostaining.

Hematoxylin & Eosin staining: Eyeballs collected from mice after sacrifice were immediately fixed with 4% paraformaldehyde for 30 min at 4 °C, embedded in OCT Compound (SAKURA, Tissue-Tek, Torrance, CA), frozen at −80 °C, cut into sections (5 mm thick) with a cryotome (CM 1850UV, Leica Microsystems AG, Wetzlar, Germany), mounted on glass slides, and stored at −80 °C. Morphological assessments of the cornea between *PPARα*$^{-/-}$ mice and WT mice were performed using Hematoxylin & Eosin (H&E) staining.

Immunofluorescence staining: For immunofluorescence staining, sections were fixed in cold acetone (−20 °C) for 10 min followed by washing them 3 times for 5 min each using PBS. Sections were then allowed to dry and 0.2% Triton X-100 was added for 20 min. After that, they were washed with PBS 3 times for 5 min each. After preincubation with 2% BSA in PBS for 60 min to block the nonspecific sites, sections were incubated at 4 °C overnight with antibodies of PPARα (1:200, ab8934, Abcam, Cambridge, UK), Ki67 (1:300, ab16667, Abcam), PMN (1:300, 20R-PR020, Fitzgerald, MA), CD45 (1:200, sc-52491, Santa Cruz). This was followed by washing them with PBS 3 times for 10 min each and incubating them with the Alexa Fluor 488-conjugated IgG (1:300, A21206, Invitrogen, Eugene, OR) or Alexa Fluor 594-conjugated IgG (1:300, A11058, Invitrogen) for 60 min at 37 °C. All sections were rinsed, counterstained with DAPI (H-1200, Vector, Burlingame, CA), and then mounted with cover slides for analysis under the confocal laser scanning microscope (Fluoview 1000, Olympus, Japan).

RNA isolation and quantitative real-time RT–PCR analysis: Total RNA was isolated from the corneal epithelium using a PicoPure RNA isolation kit (KIT0204, Arcturus, Mountain View, CA). Six to eight samples per group were used, and each sample contained pooled corneal epithelium from two mice. cDNA was synthesized using a reverse transcription kit (RR047A, TaKaRa, Shiga, Japan). Real-time PCR was

performed using a StepOne Real-Time PCR detection System (Applied Biosystems, Alameda, CA) with the use of a SYBR Premix Ex Taq Kit (RR420A, TaKaRa) as per the manufacturer's instructions. The primer sequences used to amplify specific gene products are listed in Table 1. The amplification program included an initial denaturation step at 95 °C for 10 min, which was followed by denaturation at 95 °C for 10 s and annealing and extending at 60 °C for 30 s for 40 cycles. The results of relative quantitative real-time PCR (qRT-PCR) were analyzed using the comparative threshold cycle (Ct) method, which was normalized with β-actin as an endogenous reference and calibrated against the normal control group.

Cell culture and scratch assay: Human corneal epithelial cell (HCEC) lines, obtained from RIKEN Biosource Center (Tokyo, Japan), were cultivated in 12-well plates until confluence. To create standardized wounds, a sterile 10 µl pipette tip was used to produce consistent, cell-free areas across each well. The wells were then rinsed with PBS three times, and the culture medium was substituted with DMEM/F12 basal medium that was supplemented with 25 µM fenofibrate, as had been previously reported [15,16]. For comparison, control dishes underwent the same procedure with scratches made, but they were cultured in medium without fenofibrate addition. After 12 and 24 h of culture, each well was meticulously photographed to capture at least four images of the scraped area. The experiment was repeated three times independently, with three wells dedicated to each culture condition. Measurements were consistently taken at the same scratched area for each time point in the study.

Statistical analysis: Data analysis for this study was performed using GraphPad Prism 6.0 software (GraphPad Software Inc., San Diego, CA). A Mann–Whitney test was employed, and a p value of less than 0.05 was considered to be statistically significant.

RESULTS

PPARα expression during corneal epithelial wound healing: To determine the function of PPARα in corneal epithelial wound healing, we investigated the temporal dynamics of PPARα expression during the process of corneal epithelial repair through immunofluorescent staining and qRT-PCR. The results demonstrated a significant increase in PPARα expression over the course of corneal epithelial repair (twofold increase at 12 h, 3.8-fold increase at 24 h), with peak levels observed at 36 h post injury (5.3-fold increase, as shown in Figure 1A–C). Subsequently, a gradual decrease of PPARα expression was observed, which suggests a potential regulatory role of PPARα in corneal epithelial wound healing (Figure 1A–C).

Characteristic of PPARα$^{-/-}$ mice: H&E staining showed no significant differences in corneal morphology between *PPARα*$^{-/-}$ mice and WT mice at the age of 10 weeks (Figure 2A). The mRNA level of PPARα significantly decreased by 83% (Figure 2B), whereas PPARβ expression was increased 1.5-fold in the corneal epithelium of *PPARα*$^{-/-}$ mice compared with those of WT mice (Figure 2C). PPARγ was seen to barely be expressed in the corneal epithelium (data not shown).

Corneal epithelial wound healing in PPARα$^{-/-}$ mice: We performed corneal epithelium scraping damage on *PPARα*$^{-/-}$ mice and WT mice and observed their wound healing at different time points after surgery. Slit lamp microscopy examinations revealed a smaller fluorescein staining area in WT mice than *PPARα*$^{-/-}$ mice at 12, 24, and 36 h post surgery. The corneal fluorescein staining demonstrated complete epithelial healing in WT mice at 36 h after injury. In contrast, *PPARα*$^{-/-}$ mice continued to exhibit positive staining even 36 h after injury, which indicates a delayed repair process. Full wound closure in *PPARα*$^{-/-}$ mice was achieved 48 h post injury (Figure 3A, B). These results indicate that PPARα is involved in the corneal epithelial repair process.

The effect of PPARα on cell proliferation during corneal epithelium repair: Ki67 is a cellular proliferation marker [17]. Immunofluorescent staining of Ki67 revealed differential corneal epithelial proliferation patterns between WT and *PPARα*$^{-/-}$ mice after injury. At 12 and 24 h after injury, the number of Ki67-positive cells in WT mice demonstrated a 1.4-fold and 1.5-fold increase compared to *PPARα*$^{-/-}$ mice (Figure 4A, B). This observation suggests that the corneal epithelium in the early stages of WT mice injury exhibits a more rapid proliferative response, which potentially contributes to a faster repair process. At 36 h after injury, the Ki67-positive cells in *PPARα*$^{-/-}$ mice had a 1.5-fold increase compared to WT mice (Figure 4A, B). This expression pattern was further validated by a qRT-PCR analysis (Figure 4C). Collectively, these findings indicated a delayed proliferative response in *PPARα*$^{-/-}$ mice compared to their WT counterparts, which suggests that the absence of PPARα may influence corneal epithelial cell proliferation during the repair process.

Inflammatory response in PPARα$^{-/-}$ mice during corneal epithelium repair: PMN stands as a commonly used biomarker to identify and characterize neutrophils [14]. Immunofluorescence staining of PMN demonstrated that at 12 h post-injury, PMN-positive cells in WT mice demonstrated a significant increase, surpassing the count in *PPARα*$^{-/-}$ mice. This indicated an enhanced early inflammatory response in the WT mice. Subsequently, at later time points of 24, 36, and 48 h, PMN-positive cells dramatically decreased in the

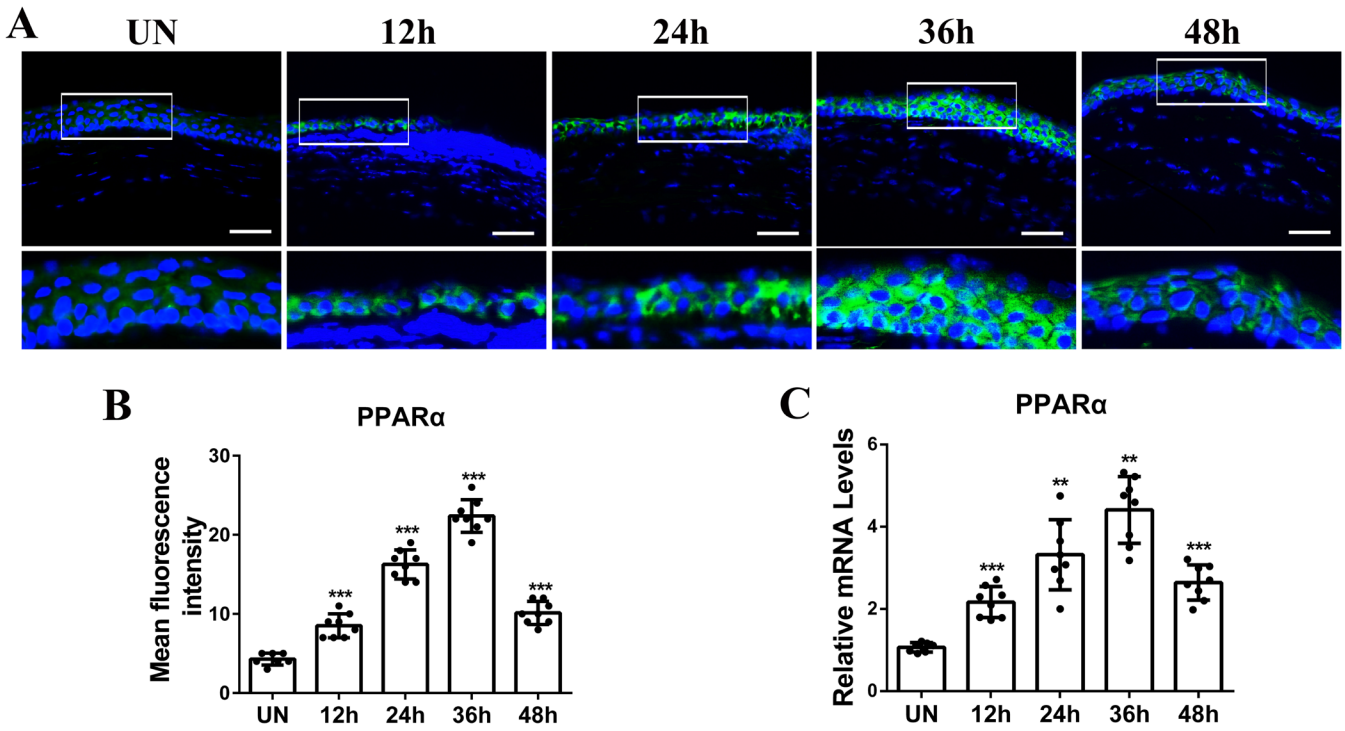

Figure 1. PPARα expression during corneal epithelial wound healing. Representative immunofluorescence staining (**A**), intensity analysis (**B**), and mRNA expression (**C**) shows a significant increase in PPARα during corneal epithelial repair. Data are shown as mean ± SD n=6–8, **p<0.01, ***p<0.001. Scale bars: 50 µm. UN, Uninjured.

corneal stroma of WT mice, while neutrophils increased in *PPARα*$^{-/-}$ mice (Figure 5A, C). The results suggested a more rapid resolution of the inflammatory response in WT mice compared to the *PPARα*$^{-/-}$ mice. Interestingly, PMN-positive cells in *PPARα*$^{-/-}$ mice demonstrated a distinct pattern. After an initial increase at 12 h, the number of PMN-positive cells continued to rise, reaching a peak at 36 h post injury. They then slightly decreased at 48 h with a substantial number of PMN-positive cells present, indicating a prolonged, delayed inflammatory response in *PPARα*$^{-/-}$ mice after corneal epithelial wounding (Figure 5A, C).

CD45, also known as leukocyte common antigen, is a widely used biomarker for leukocytes, which play a significant

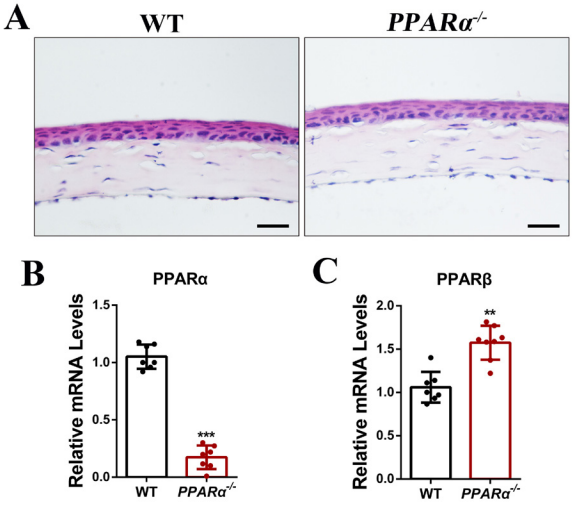

Figure 2. Characteristic of *PPARα*$^{-/-}$ mic. Representative H&E staining shows corneal morphology of *PPARα*$^{-/-}$ mice and WT mice (**A**). Gene expression level of PPARα significantly decreased in *PPARα*$^{-/-}$ mice (**B**). Gene expression level of PPARβ significantly increased in *PPARα*$^{-/-}$ mice (**C**). Data are shown as mean ± SD n=6–8, **p<0.01, ***p<0.001. Scale bars: 100 µm.

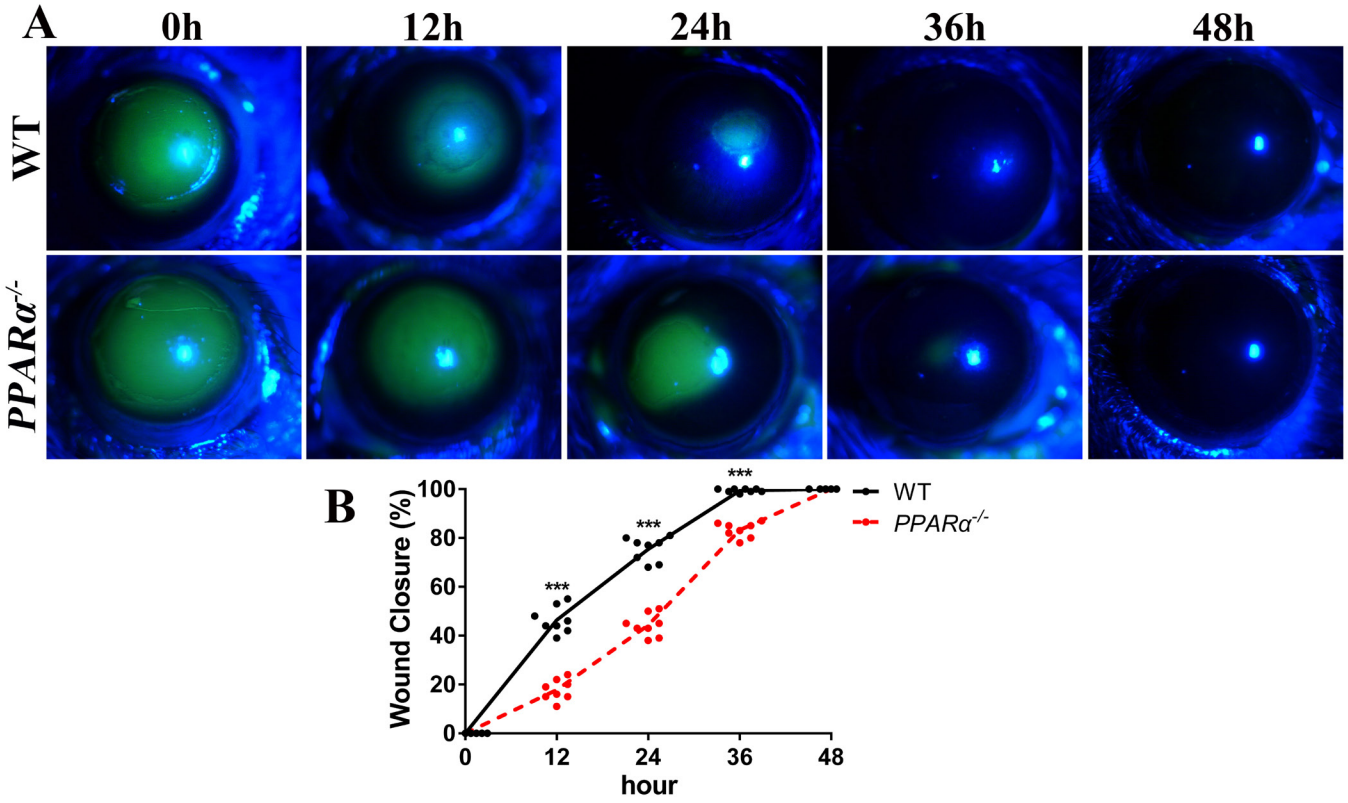

Figure 3. Corneal epithelial wound healing in *PPARα*$^{-/-}$ mice. Representative slit-lamp images (**A**) and wound closure analysis (**B**) show the fluorescein staining area in WT mice compared to *PPARα*$^{-/-}$ mice at different time points (0/12/24/36/48 h). Data are shown as mean ± SD n=6–8, ***p<0.001.

role in the inflammatory response [18]. Immunofluorescence staining of CD45 found that at 12 and 24 h post injury, the number of CD45-positive cells in WT mice had a 1.4-fold and 1.3-fold increase compared to *PPARα*$^{-/-}$ mice, which indicates a heightened early inflammatory response in the WT mice. Subsequently, after 24 h, the number of CD45-positive cells in WT mice demonstrated a significant decrease, reaching notably lower levels than *PPARα*$^{-/-}$ mice at 36 h. There were basically no CD45-positive cells in WT mice 48 h after injury (Figure 5B, D). Conversely, in *PPARα*$^{-/-}$ mice, the number of CD45-positive cells continued to rise, peaking at 36 h post injury. A slight decline was observed 48 h after injury, and a substantial number of CD45-positive cells remained in the corneal stroma. This further confirmed a delayed and prolonged inflammatory response in *PPARα*$^{-/-}$ mice (Figure 5B, D). These findings suggest that corneal inflammatory responses were exaggerated and delayed during wound healing in the absence of PPARα.

PPARα agonist promotes corneal epithelial wound healing in WT mice but not PPARα$^{-/-}$ *mice:* To further determine the effect of PPARα on in vivo corneal epithelial wound healing, PPAR agonist fenofibrate solution was topically applied on the ocular surface of the WT mice after corneal epithelial scraping. Slit lamp images showed that fenofibrate administration led to a marked acceleration of corneal epithelial repair, with near-complete corneal epithelial restoration observed 24 h post treatment (Figure 6A, B). After fenofibrate application for 12 h, immunofluorescence staining and qRT-PCR analysis demonstrated a significant increase in PPARα expression in the corneal epithelium (Figure 6C–E). This result indicated the effective activation of the PPARα pathway by fenofibrate treatment. Moreover, the Ki67-positive cell and mRNA expression of Ki67 further exhibited a substantial increase following fenofibrate administration (Figure 6F–H). This observation suggests that fenofibrate may stimulate the proliferation of corneal epithelial cells, thus promoting corneal epithelial repair. However, the absence of substantial improvements in corneal epithelial repair in *PPARα*$^{-/-}$ mice treated with fenofibrate provided additional evidence supporting the idea that fenofibrate aids in promoting corneal epithelial repair specifically through PPARα activation (Figure 7A, B).

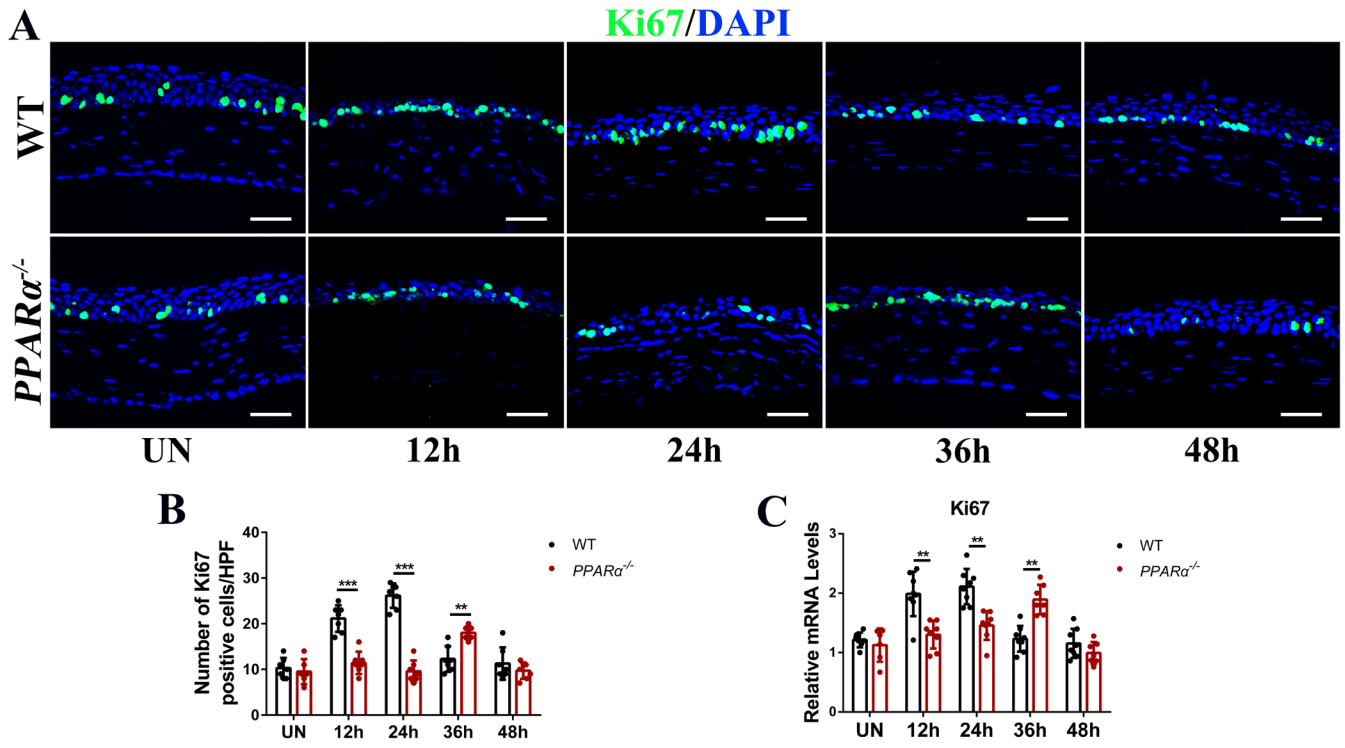

Figure 4. The effect of PPARα on cell proliferation during corneal epithelium repair. Immunofluorescence staining of Ki67 (**A**), positive cell counting (**B**), and mRNA expression (**C**) shows a more rapid cellular proliferation in WT mice compared to *PPARα*$^{-/-}$ mice during corneal epithelial repair. Data are shown as mean ± SD n=6–8, **p<0.01, ***p<0.001. Scale bars: 50 µm. UN, Uninjured.

PPARα agonist promotes cornea epithelial wound healing in vitro: To further investigate the impact of fenofibrate on corneal epithelial cell wound healing in an in vitro model, a scratch assay was conducted using an HCEC culture assay. The results revealed that within 24 h, the wound area was essentially closed in the fenofibrate-treated group, while there were unhealed regions in the control group (Figure 8A, B). These results indicated that fenofibrate could promote wound healing of corneal epithelial cells in vitro.

DISCUSSION

Our study provides new insights into the role of PPARα in corneal epithelial wound healing. Through utilization of PPARα gene-knockout mice, we demonstrated that an absence of PPARα results in delayed corneal epithelial wound repair. We further confirmed that PPARα regulates the proliferation of corneal epithelial cells.

The temporal dynamics of PPARα expression during corneal epithelial repair revealed a pronounced upregulation of PPARα, which peak at 36 h post injury. These observations suggest that PPARα may play a pivotal role in the initial response to corneal injury, potentially facilitating repair machinery activation. The observed alterations in corneal epithelial wound healing in *PPARα*$^{-/-}$ mice further supported the role of PPARα in this repair process.

PPARα and PPARβ exhibit similar functions in certain tissues, which could potentially lead to compensatory effects [19,20]. Leone et al. found that in *PPARα*$^{-/-}$ mice, fasting-induced upregulation of several PPAR-responsive genes in the liver and heart is attenuated but not completely blunted. This suggests the presence of residual effects or partial compensation involving PPARβ [21]. However, in certain tissues, PPARα loss does not induce PPARβ expression change. Liu et al. reported that PPARβ remained unaltered in the heart of *PPARα*$^{-/-}$ mice [22]. In our current study, *PPARα*$^{-/-}$ mice demonstrated downregulation of PPARα expression and compensatory upregulation of PPARβ expression. It is conceivable that corneal PPARβ might not be indispensable for corneal wound healing, or its contribution could be relatively subdued. Nevertheless, the underlying mechanisms driving PPARβ upregulation warrant further in-depth investigations.

Corneal epithelial healing occurs through a multifaceted, intricate process shaped by the convergence of interconnected

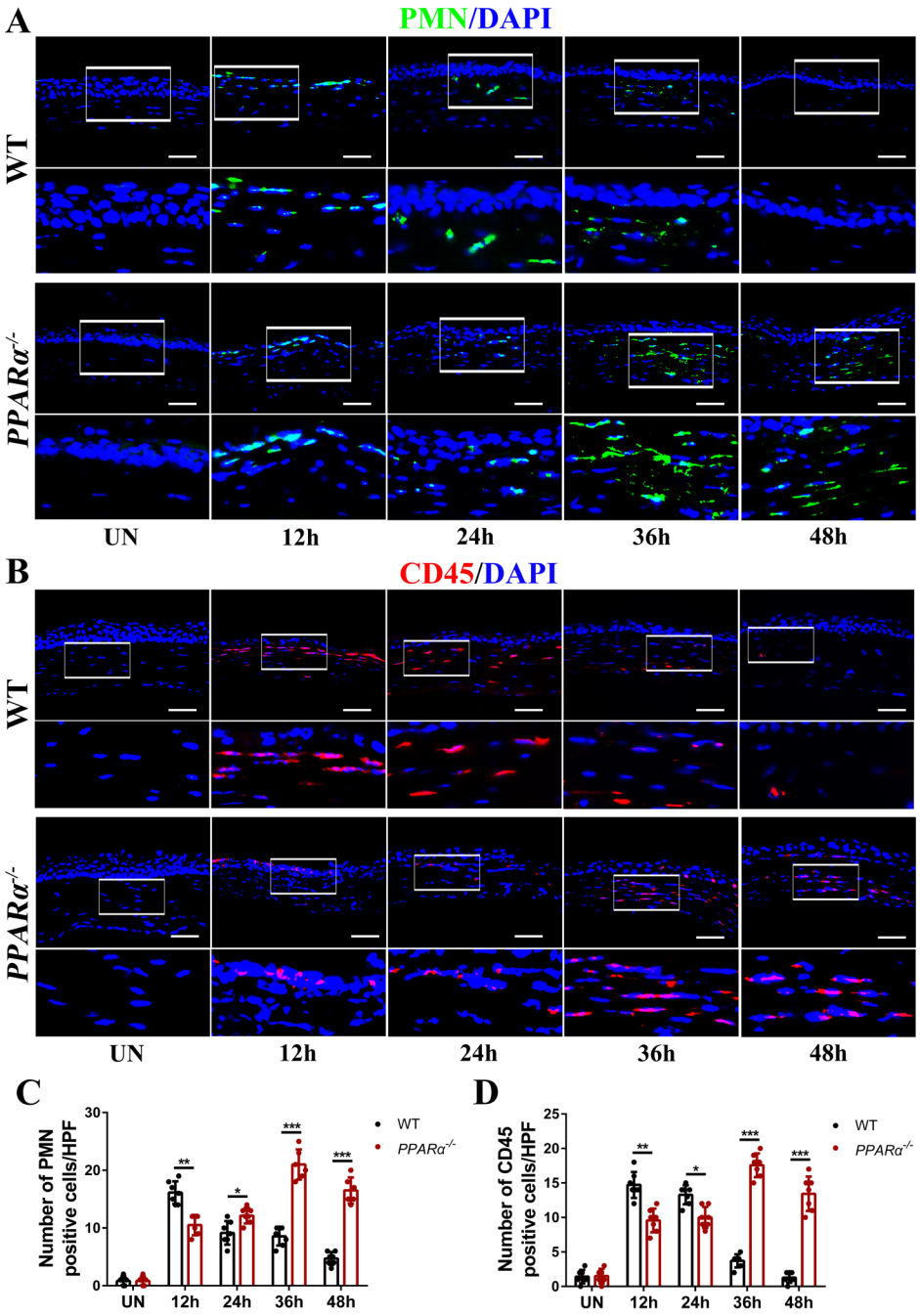

Figure 5. Inflammatory response in PPARα−/− mice during corneal epithelium repair. Immunofluorescence staining of PMN (**A**) and positive cell counting (**C**) shows more positive cells at 12 h post injury and then dramatically decreases in WT mice compared to *PPARα*$^{-/-}$ mice. Immunofluorescence staining of CD45 **(B)** and positive cell counting **(D)** shows more positive cells at 12 and 24 h post injury and then dramatically decreases in WT mice compared to *PPARα*$^{-/-}$ mice. Data are shown as mean ± SD n=6–8, *p<0.05, **p<0.01, ***p<0.001. Scale bars: 50 µm. UN, Uninjured.

events, such as cell proliferation and migration and the inflammatory response to injury. The inflammatory response in corneal epithelial repair is a critical aspect of the healing process [23-25]. Following injury to the cornea, there is a notable influx of inflammatory cells into the corneal stroma, which typically manifests between 12 to 24 h post injury [26]. The primary function of these inflammatory cells at the wound site is to scavenge cellular components released

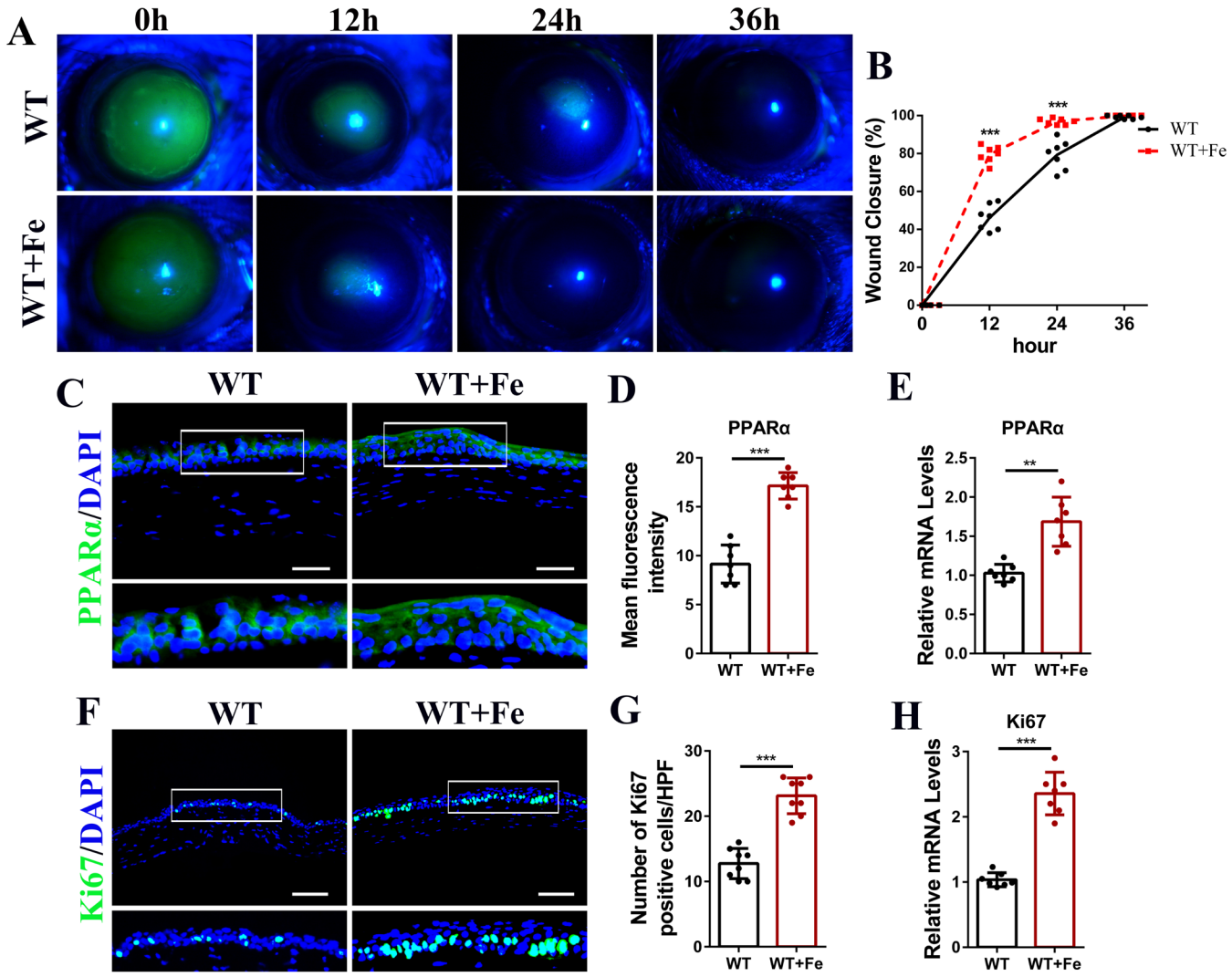

Figure 6. PPARα agonist promotes corneal epithelial wound healing in WT mice. Representative slit-lamp images (**A**) and wound closure analysis (**B**) show that fenofibrate promotes corneal epithelial repair. Representative immunofluorescence staining (**C**), intensity analysis (**D**), and mRNA expression (**E**) shows that fenofibrate increases PPARα expression during corneal epithelial repair. Representative immunofluorescence staining (**F**), intensity analysis (**G**), and mRNA expression (**H**) demonstrate that fenofibrate increases Ki67 expression during corneal epithelial repair. Data are shown as mean ± SD n=6–8, **p<0.01, ***p<0.001. Scale bars: 50 µm (C), Scale bars: 100 µm (F). Fe, Fenofibrate.

from keratocytes that undergo programmed cell death [27]. Previous studies have provided evidence to support the critical role of neutrophil emigration in facilitating efficient corneal re-epithelialization [28]. Inhibition of neutrophil migration into the cornea following epithelial abrasion significantly prolongs the healing time by 12 to 24 h [28]. The beneficial effects of neutrophils on the re-epithelialization process may be attributed to various mechanisms. First, neutrophils are capable of delivering crucial growth factors that support the corneal epithelium's regenerative processes [29]. These growth factors are known to stimulate cell proliferation and migration, facilitating rapid wound closure [29]. Additionally, neutrophils could kill pathogens and create a favorable environment for efficient re-epithelialization to occur [30]. In the skin, PPARα is primarily involved in the early inflammation phase of healing. The neutrophils recruitment to the wound bed is impaired in *PPARα*$^{-/-}$ mice during the very early inflammatory phase [11]. Our findings reveal distinct patterns of neutrophil infiltration in WT and *PPARα*$^{-/-}$ mice during corneal wound healing. WT mice displayed an enhanced early neutrophil accumulation followed by rapid resolution, while *PPARα*$^{-/-}$ mice exhibited delayed neutrophil

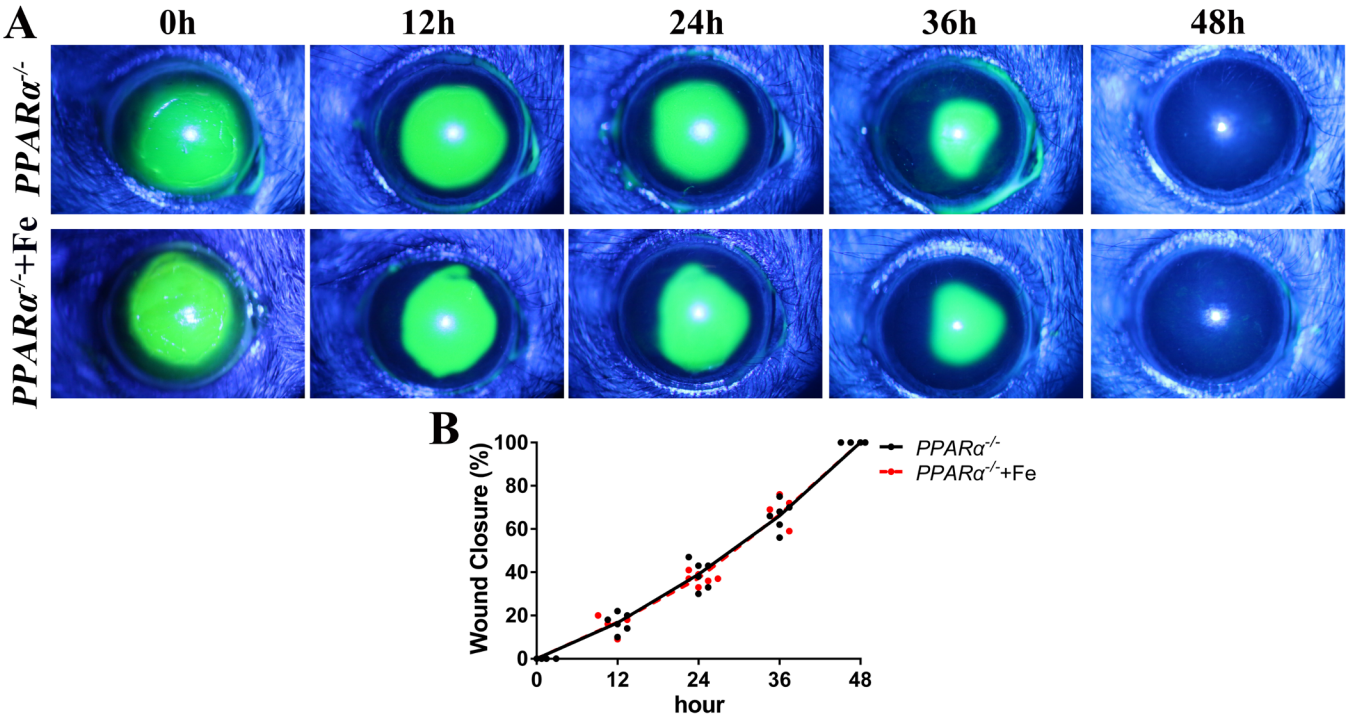

Figure 7. PPARα agonist has no effect on corneal epithelial wound healing in *PPARα*$^{-/-}$ mice. Representative slit-lamp images **(A)** and wound closure analysis **(B)** show that fenofibrate does not promote corneal epithelial repair in *PPARα*$^{-/-}$ mice. Data are shown as mean ± SD n=6. Fe, Fenofibrate.

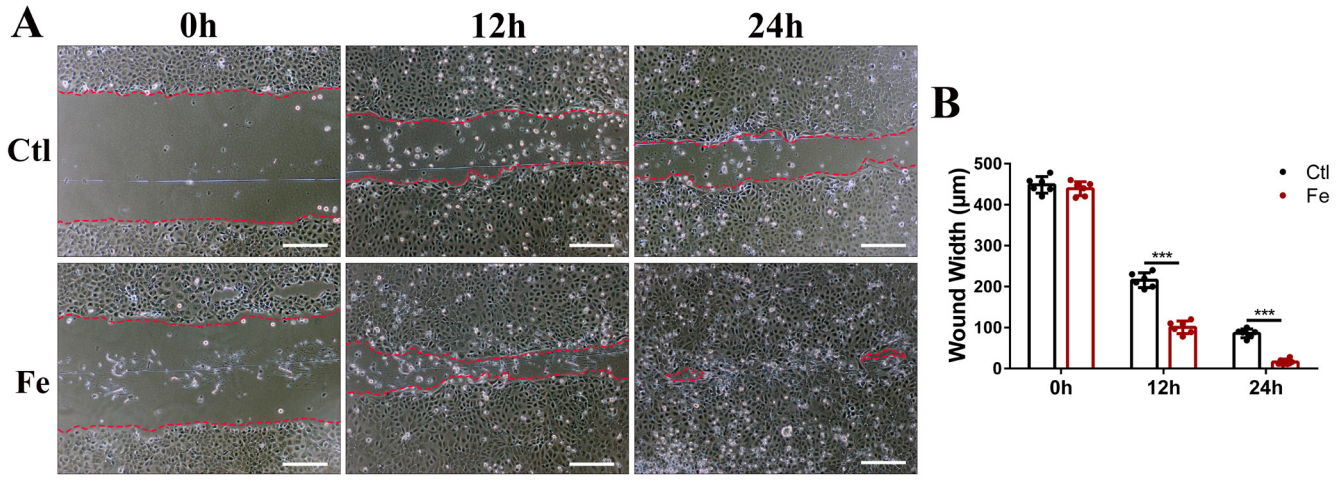

Figure 8. PPARα agonist promotes corneal epithelial migration capacity in vitro. Representative slit-lamp images (**A**) and wound width analysis (**B**) show that fenofibrate promotes migration and the wound healing capability of corneal epithelial cells in vitro. Data are shown as mean ± SD n=6, ***p<0.001. Scale bars: 200 μm. Ctl, Control. Fe, Fenofibrate.

accumulation. These observations suggest that PPARα may modulate the inflammatory process during corneal wound healing, influence the recruitment and resolution of inflammatory cells, and alter the corneal wound healing process.

Cell proliferation is an important process that occurs to repopulate the corneal wound area. The reduced number of Ki67-positive cells in *PPARα*$^{-/-}$ mice at 12 and 24 h after injury implies a delayed early-stage proliferative response. At 36 h post-injury, *PPARα*$^{-/-}$ mice demonstrated a notable surge in Ki67-positive cells, surpassing the levels observed in WT mice. The observed delayed early-stage proliferative response in *PPARα*$^{-/-}$ mice aligned with their slower wound healing process, as earlier proliferative events play a crucial role in the initiation of corneal re-epithelialization.

The use of PPARα agonist fenofibrate provided further evidence supporting the involvement of PPARα in corneal epithelial wound healing. Fenofibrate administration resulted in a marked acceleration of corneal epithelial repair in WT mice, with near-complete restoration observed 24 h post-treatment. The upregulation of PPARα expression and increased proliferation in response to fenofibrate treatment further supported the notion that PPARα plays an important role in promoting efficient corneal epithelial repair. Moreover, in vitro studies using HCECs demonstrated that fenofibrate enhanced corneal epithelial cell wound healing in an in vitro model. This finding suggested that PPARα activation may promote the wound healing capability of corneal epithelial cells, supporting the in vivo observations in the murine model.

In conclusion, our results indicated that PPARα is an essential regulator of corneal epithelial wound healing. The upregulation of PPARα expression during the repair process, the altered proliferative response, and the distinct patterns of inflammatory infiltrate in *PPARα*$^{-/-}$ mice all point to the multifaceted role of PPARα during corneal wound healing. The use of a PPARα agonist further highlighted the potential therapeutic implications of PPARα modulation in promoting more efficient corneal epithelial repair. However, further research is required to fully elucidate the molecular pathways through which PPARα exert its effects in promoting corneal epithelial wound healing.

ACKNOWLEDGMENTS

This study was supported in part by the National Natural Science Foundation of China (NSFC, No. 82,000,856 [to YW], No. 82,000,868 [to JB], No. 81,970,773 [to WL]), the Natural Science Foundation of Fujian Province/ Fujian Provincial Health Technology Project (No. 2020D038 [to YW]), Xiamen University Nanqiang Outstanding Talents Program (JB). The funders have no role in the study design, data collection and analysis, decision on publishing, or preparation of the manuscript. Author contributions statement: Y.W, J.B and W.L designed the experiments; Y.W, J.B, L.S and R.Z performed experiments and interpreted data; Y.W, J.B, Y.Y and W.L wrote and revised the manuscript. All authors critiqued and approved the manuscript. Competing Interests: The authors have declared that no competing interests exist.: Dr. Wei Li (wei1018@xmu.edu.cn) and Dr. Yuanzhi Yuan (yuan.yuanzhi@zs-hospital.sh.cn) are co-corresponding authors for this paper.

REFERENCES

1. Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev 1999; 20:649-88. [PMID: 10529898].
2. Manickam R, Duszka K, Wahli W. PPARs and Microbiota in Skeletal Muscle Health and Wasting. Int J Mol Sci 2020; 21:8056-[PMID: 33137899].
3. Manickam R, Duszka K, Wahli W. PPARs and Microbiota in Skeletal Muscle Health and Wasting. Int J Mol Sci 2020; 21:8056-[PMID: 33137899].
4. Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 1990; 347:645-50. [PMID: 2129546].
5. Braissant O, Foufelle F, Scotto C, Dauça M, Wahli W. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. Endocrinology 1996; 137:354-66. [PMID: 8536636].
6. Bookout AL, Jeong Y, Downes M, Yu RT, Evans RM, Mangelsdorf DJ. Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. Cell 2006; 126:789-99. [PMID: 16923397].
7. Cunard R, DiCampli D, Archer DC, Stevenson JL, Ricote M, Glass CK, Kelly CJ. WY14,643, a PPAR alpha ligand, has profound effects on immune responses in vivo. J Immunol 2002; 169:6806-12. [PMID: 12471112].
8. Choi JM, Bothwell AL. The nuclear receptor PPARs as important regulators of T-cell functions and autoimmune diseases. Mol Cells 2012; 33:217-22. [PMID: 22382683].
9. Pineda Torra I, Gervois P, Staels B. Peroxisome proliferator-activated receptor alpha in metabolic disease, inflammation, atherosclerosis and aging. Curr Opin Lipidol 1999; 10:151-9. [PMID: 10327283].
10. Icre G, Wahli W, Michalik L. Functions of the peroxisome proliferator-activated receptor (PPAR) alpha and beta in skin homeostasis, epithelial repair, and morphogenesis. J Investig Dermatol Symp Proc 2006; 11:30-5. [PMID: 17069008].
11. Michalik L, Desvergne B, Tan NS, Basu-Modak S, Escher P, Rieusset J, Peters JM, Kaya G, Gonzalez FJ, Zakany J, Metzger D, Chambon P, Duboule D, Wahli W. Impaired skin

wound healing in peroxisome proliferator-activated receptor (PPAR)alpha and PPARbeta mutant mice. J Cell Biol 2001; 154:799-814. [PMID: 11514592].

12. Zhou T, Yan K, Zhang Y, Zhu L, Liao Y, Zheng X, Chen Y, Li X, Liu Z, Zhang Z. Fenofibrate suppresses corneal neovascularization by regulating lipid metabolism through PPARα signaling pathway. Front Pharmacol 2022; 13:1000254[PMID: 36588740].
13. Liang W, Huang L, Whelchel A, Yuan T, Ma X, Cheng R, Takahashi Y, Karamichos D, Ma JX. Peroxisome proliferator-activated receptor-α (PPARα) regulates wound healing and mitochondrial metabolism in the cornea. Proc Natl Acad Sci U S A 2023; 120:e2217576120[PMID: 36943878].
14. Bu J, Wu Y, Li K, Zhang M, Zhang R, Sun L, Guo Y, He H, Li S, Liu Z, Li W. Transitory alkali exposure on meibomian gland orifices induces meibomian gland dysfunction. Ocul Surf 2023; 29:406-15. [PMID: 37327868].
15. Tang L, Wang X, Wu J, Li SM, Zhang Z, Wu S, Su T, Lin Z, Chen X, Liao X, Bai T, Qiu Y, Reinach PS, Li W, Chen Y, Liu Z. Sleep Deprivation Induces Dry Eye Through Inhibition of PPARα Expression in Corneal Epithelium. Invest Ophthalmol Vis Sci 2018; 59:5494-508. [PMID: 30658033].
16. Wang X, Tang L, Zhang Z, Li W, Chen Y. Keratocytes promote corneal neovascularization through VEGFr3 induced by PPARα-inhibition. Exp Eye Res 2020; 193:107982[PMID: 32092288].
17. Bu J, Wu Y, Cai X, Jiang N, Jeyalatha MV, Yu J, He X, He H, Guo Y, Zhang M, Quantock AJ, Liu Z, Li W. Hyperlipidemia induces meibomian gland dysfunction. Ocul Surf 2019; 17:777-86. [PMID: 31201956].
18. Bu J, Zhang M, Wu Y, Jiang N, Guo Y, He X, He H, Jeyalatha MV, Reinach PS, Liu Z, Li W. High-Fat Diet Induces Inflammation of Meibomian Gland. Invest Ophthalmol Vis Sci 2021; 62:13-[PMID: 34398199].
19. Yang Q, Li Y. Roles of PPARs on regulating myocardial energy and lipid homeostasis. J Mol Med (Berl) 2007; 85:697-706. [PMID: 17356846].
20. Gilde AJ, van der Lee KA, Willemsen PH, Chinetti G, van der Leij FR, van der Vusse GJ, Staels B, van Bilsen M. Peroxisome proliferator-activated receptor (PPAR) alpha and PPARbeta/delta, but not PPARgamma, modulate the expression of genes involved in cardiac lipid metabolism. Circ Res 2003; 92:518-24. [PMID: 12600885].
21. Leone TC, Weinheimer CJ, Kelly DP. A critical role for the peroxisome proliferator-activated receptor alpha (PPARalpha) in the cellular fasting response: the PPARalpha-null mouse as a model of fatty acid oxidation disorders. Proc Natl Acad Sci U S A 1999; 96:7473-8. [PMID: 10377439].
22. Liu J, Wang P, He L, Li Y, Luo J, Cheng L, Qin Q, Brako LA, Lo WK, Lewis W, Yang Q. Cardiomyocyte-Restricted Deletion of PPARβ/δ in PPARα-Null Mice Causes Impaired Mitochondrial Biogenesis and Defense, but No Further Depression of Myocardial Fatty Acid Oxidation. PPAR Res 2011; 372854:.
23. Zieske JD, Guimarães SR, Hutcheon AE. Kinetics of keratocyte proliferation in response to epithelial debridement. Exp Eye Res 2001; 72:33-9. [PMID: 11133180].
24. West-Mays JA, Sadow PM, Tobin TW, Strissel KJ, Cintron C, Fini ME. Repair phenotype in corneal fibroblasts is controlled by an interleukin-1 alpha autocrine feedback loop. Invest Ophthalmol Vis Sci 1997; 38:1367-79. [PMID: 9191600].
25. Zhu SN, Dana MR. Expression of cell adhesion molecules on limbal and neovascular endothelium in corneal inflammatory neovascularization. Invest Ophthalmol Vis Sci 1999; 40:1427-34. [PMID: 10359324].
26. O'Brien TP, Li Q, Ashraf MF, Matteson DM, Stark WJ, Chan CC. Inflammatory response in the early stages of wound healing after excimer laser keratectomy. Arch Ophthalmol 1998; 116:1470-4. [PMID: 9823348].
27. Wilson SE, Mohan RR, Mohan RR, Ambrósio R Jr, Hong J, Lee J. The corneal wound healing response: cytokine-mediated interaction of the epithelium, stroma, and inflammatory cells. Prog Retin Eye Res 2001; 20:625-37. [PMID: 11470453].
28. Li Z, Burns AR, Smith CW. Two waves of neutrophil emigration in response to corneal epithelial abrasion: distinct adhesion molecule requirements. Invest Ophthalmol Vis Sci 2006; 47:1947-55. [PMID: 16639002].
29. Chahud F, Ramalho LN, Ramalho FS, Haddad A, Roque-Barreira MC. The lectin KM+ induces corneal epithelial wound healing in rabbits. Int J Exp Pathol 2009; 90:166-73. [PMID: 19335555].
30. Wolf M, Maltseva I, Clay SM, Pan P, Gajjala A, Chan MF. Effects of MMP12 on cell motility and inflammation during corneal epithelial repair. Exp Eye Res 2017; 160:11-20. [PMID: 28442300].