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Basic Study
Regulation of PPAR-γ activity in lipid-laden hepatocytes affects macrophage polarization and inflammation in NAFLD

Lipid-laden hepatocytes affect macrophages

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Abstract

BACKGROUND
Lipid metabolism disorder and inflammatory-immune activation are vital triggers in the pathogenesis of NAFLD. Various studies have shown that PPAR-γ exerts potent anti-inflammatory and immunomodulatory properties. However, little is known about regulation of PPAR-γ activity in modulating cells cross-talk in NAFLD.

AIM
To investigate whether the regulation of PPAR-γ activity in lipid-laden hepatocytes affects macrophage polarization and inflammation.

METHODS
Primary hepatocytes were isolated from wild-type C57BL6/J mice or hepatocyte-specific PPAR-γ knockout mice and incubated with free fatty acids (FFAs). Macrophages were incubated with conditioned medium (CM) from lipid-laden hepatocytes with or without the PPAR-γ agonist. Wild-type C57BL/6J mice were fed a high-fat diet and administered rosiglitazone.

RESULTS
Primary hepatocytes exhibited significant lipid deposition and increased ROS production after FFA incubation. Conditioned medium from lipid-laden hepatocytes promoted macrophage polarization to the M1 type and activation of the TLR4/NF-κB pathway. A PPAR-γ agonist ameliorated oxidative stress and NLRP3 inflammasome activation in lipid-laden hepatocytes and subsequently prevented M1 macrophage polarization. Hepatocyte-specific PPAR-γ deficiency aggravated oxidative stress and NLRP3 inflammasome activation in lipid-laden hepatocytes, which further promoted M1 macrophage polarization. Rosiglitazone administration improved oxidative stress and NLRP3 inflammasome activation in HF diet-induced NAFLD mice in vivo.
CONCLUSION

Up-regulation of PPAR-γ activity in hepatocytes alleviated NAFLD by modulating the cross-talk between hepatocytes and macrophages via the ROS-NLRP3-IL-1β pathway.

**Key Words:** Key words: Nonalcoholic fatty liver disease; Hepatocyte; Macrophage polarization; PPAR-γ; NLRP3; Oxidative stress

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**Core Tip:** Nonalcoholic fatty liver disease (NAFLD) is currently one of the most epidemic chronic liver diseases worldwide. We aimed to investigate whether the regulation of PPAR-γ activity in lipid-laden hepatocytes affects macrophage polarization and explore the underlying mechanism. Our study revealed that lipid-laden hepatocytes skewed macrophages polarization to M1 phenotype. Regulation of PPAR-γ activity alleviates NAFLD through modulating the cross-talk between hepatocytes and macrophages via the ROS-NLRP3-IL-1β signaling pathway. Strategies that manipulation of PPAR-γ activity to regulate cells cross-talk will be beneficial for treating NAFLD.

**INTRODUCTION**

Nonalcoholic fatty liver disease (NAFLD) is currently one of the most common liver diseases, with a high morbidity presently exceeding 25% worldwide[1-2]. Manifesting from simple hepatic steatosis to nonalcoholic steatohepatitis (NASH) and even to cirrhosis and hepatocellular carcinoma, NAFLD has already posed heavy public and financial burdens worldwide [3]. Given the lack of effective medications for treatment, there is a need to deeply explore the pathogenesis of NAFLD and to look for potential therapeutic targets for alleviating NAFLD[4].
Lipid metabolism disorder and inflammatory immune activation are two main triggers in the pathogenesis of NAFLD\[^3\]. The widely accepted “two-hit theory” suggests that excess free fatty acids (FFAs) act as the first hit, causing abnormal lipid accumulation and insulin resistance, increasing the susceptibility of the liver to inflammatory damage\[^5, 6\]. Based on the first hit, the second hit involves activation of immune cells and oxidative metabolite production, leading to oxidative stress and an inflammatory response\[^7-9\]. Hence, the accumulation of lipotoxic agents in hepatocytes is key to the onset and progression of NAFLD. Lipotoxicity can directly induce endoplasmic reticulum stress and pyroptosis in steatotic hepatocytes\[^10, 11\]. Among the many factors that trigger the progression of NAFLD to NASH, activation of immune cells exerts a prominent and indispensable role\[^12\].

Activation of macrophages, including hepatic resident Kupffer cells and peripherally recruited monocytes, plays an important role in the progression of NAFLD\[^12, 13\]. It is now widely considered that macrophages can be classified into two types: the classically activated M1 phenotype and the alternatively activated M2 phenotype. M1 phenotype macrophages are mainly induced by interferon-γ (IFN-γ) and lipopolysaccharide (LPS) and secrete pro-inflammatory factors (IL-1, 6, 12, 23, CXCL 10, NO, peroxides, etc.), which participate in the Th1 immune response and exert pro-inflammatory, bactericidal and anti-tumor effects. M2 macrophages are mainly induced by IL-4 and IL-10 and participate in the Th2 immune response with anti-inflammatory and tissue remodeling effects\[^14, 15\]. Under normal conditions, macrophages in the liver predominantly exhibit an M2 phenotype\[^16\]. However, in NAFLD mice induced by a high-fat diet, the number of macrophages increases dramatically, and the polarity of macrophages appears to be shifted toward the M1 type\[^17\]. These M1 phenotype macrophages contribute to the progression and prolongation of liver inflammation\[^13\]. However, there is no certainty as to what exactly drives the activation of macrophages. Recent studies have revealed that various factors, including high levels of free fatty acids and the gut microbiota, may lead to macrophage activation\[^18, 19\]. Furthermore, the
cross-talk or interaction between parenchymal and nonparenchymal cells in the liver may regulate their phenotype or function reciprocally.

PPAR-γ is a ligand-activated nuclear transcription receptor that mainly participates in adipocyte differentiation, lipogenesis, and insulin resistance[20]. Recently, much attention has been focused on the immunomodulatory and anti-inflammatory properties of PPAR-γ[21]. It has been demonstrated that activation of PPAR-γ synergistically upregulates the NRF2/HO-1 signaling pathway, thereby ameliorating methotrexate-induced hepatotoxicity[22]. Our previous study demonstrated that regulation of PPAR-γ activity in macrophages and HSCs could modulate their activation and alleviate the development of NAFLD/NASH[17, 23]. Most of the previous studies focused on the anti-inflammatory properties of PPAR-γ in non-parenchymal cells such as macrophages and HSCs in NASH, thus it remains to be explored the role of PPAR-γ in hepatocytes as well as in the interaction between hepatocytes and macrophages.

In the current study, we aimed to investigate whether the regulation of PPAR-γ activity in lipid-laden hepatocytes affects macrophage polarization and explore the underlying mechanism. We found that up-regulation of PPAR-γ activity could alleviate NAFLD through modulation of the cross-talk between hepatocytes and macrophages by the ROS-NLRP3-IL-1β signaling pathway.

MATERIALS AND METHODS

Primary hepatocyte isolation and treatment

Primary hepatocytes were isolated from wild-type C57BL/6 mice or hepatocyte-specific PPAR-γ knockout mice by a two-step collagenase in situ perfusion of the liver[24] and cultured in DMEM containing 10% FBS (Gibco, Waltham, MA, USA) with 100 U/mL penicillin G and 100 U/mL streptomycin sulfate at 37 °C with 5% CO₂ on collagen I-coated plates. The viability of primary hepatocytes was assessed by the trypan blue exclusion test and was greater than 95%. Mixed free fatty acids (FFAs) with a final concentration of 1 mmol/L were prepared with palmitic acid (PA, 0.66 mmol/L, Sigma
Aldrich) and oleic acid (OA, 0.33 mmol/L, Sigma Aldrich)\textsuperscript{[25]}.

After overnight culture, primary hepatocytes were treated with FFA for 24 h to induce a cell model of NAFLD in vitro. In some experiments, primary hepatocytes were pretreated with the PPAR-\(\gamma\) agonist GW1929 (20 \(\mu\)mol/L, Sigma Aldrich) for 3 h, followed by incubation with FFA for 6 h or 24 h. Cell lysates were collected for RT-PCR and western blot analyses.

**RAW264.7 macrophage culture and treatment**

RAW264.7 macrophages were purchased from the Cell Bank of the Chinese Academy of Sciences and cultured in DMEM containing 10% fetal bovine serum with 100 U/mL penicillin G and 100 U/mL streptomycin sulfate at 37 °C with 5% CO\(_2\). All experimental interventions were conducted on the third passage of cells.

**Primary hepatocyte and RAW264.7 macrophage conditional coculture system**

As mentioned above, primary hepatocytes were incubated with FFA or GW1929 for 3 h followed by FFA. Then, the cell culture supernatants were collected, centrifuged, and filtered to remove impurities. RAW264.7 macrophages were incubated with different conditioned media (CM) from primary hepatocytes to establish conditional coculture systems for 6 h or 24 h, which were called CM-NC, CM-FFA, and CM-GW1929+FFA, respectively.

**Animal experiments**

The animal protocol was designed to minimize pain or discomfort to the animals. Pparg\(^{a/f}\) mice and Alb-cre mice on the C57BL.6/J background were purchased from GemPharmatech (Nanjing, China) to breed and obtain hepatocyte-specific PPAR-\(\gamma\) knockout (PPAR-\(\gamma^{\text{Ahep}}\)) male mice (Figure S2). Wild-type C57BL/6 male mice (aged 6-8 wk) were obtained from the Experimental Animal Center (Renji Hospital, Shanghai Jiao Tong University). Wild-type mice were fed either a normal control (NC) diet (15% kilocalories from fat, \(n = 10\)) or a high-fat (HF) diet (60% kilocalories from fat, \(n = 10\)) for 16 wk. For the rosiglitazone intervention experiment, mice received rosiglitazone (30
mg/kg/day, Sigma Aldrich) by oral gavage once daily for 28 consecutive days after 12 wk of HF diet feeding (n = 10). All animal experiments fulfilled the Shanghai Jiao Tong University criteria for the humane treatment of laboratory animals and were approved by the Renji Hospital Animal Care and Use Committee (Permit number: RJ2018-0930).

**Oil Red O staining**
Free fatty acid-treated hepatocytes were fixed with 4% paraformaldehyde for 1 h and then stained with 5 mg/mL Oil Red O (Sigma Aldrich) for 60 min to examine lipid accumulation.

**Assay of lipid contents, IL-1β concentration and oxidative stress markers**
The cell culture supernatant of primary hepatocytes was collected for further analysis. Triglyceride (TG) and total cholesterol (T-CHO) were measured using a triglyceride assay kit and a total cholesterol assay kit, respectively (Nanjing Jiancheng Bioengineering Institute, China). The IL-1β concentration was measured using an IL-1β ELISA kit (Lianke Biotechnology Company, China). Plasma from mice was centrifuged, separated and stored at -80 °C for further analysis. Plasma levels of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH) were measured using MDA, SOD and GSH assay kits, respectively (Nanjing Jiancheng Bioengineering Institute, China). The plasma level of reactive oxygen species (ROS) was measured using an ROS ELISA kit (Nanjing JiaBeiSen Biotechnology, China). ROS generation in the cell culture supernatant was assayed using a DCFH-DA fluorescent probe kit (Beyotime Biotechnology, China). Total protein was extracted from mouse liver tissues. Caspase-1 activity in liver tissues was assessed with a Caspase-1 activity assay kit (BioVision, Milpitas, CA, United States). All procedures were performed according to the manufacturer’s instructions.

**Total RNA isolation and real-time PCR**
Total RNA was extracted from mouse liver tissues, RAW264.7 macrophages and primary hepatocytes using TRIzol reagent (TaKaRa, Kusatsu, Japan). Complementary DNA was generated from 1 μg of RNA using a cDNA synthesis kit (Nanjing Vazyme Biotech, China). For real-time PCR, 10 ng of template was added to a 10-μL reaction system containing each primer and SYBR Green PCR Master Mix (TaKaRa, Kusatsu, Japan). The PCR thermocycling parameters were 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s performed by the ABI Prism 7300 system (Applied Biosystems, Foster City, CA). All reactions were performed in triplicate. The expression levels of target genes were quantified by the double-delta method (2^{ΔΔCt}). Murine primers (provided by Sangon Biotech Co., Shanghai, China) were as follows (Table 1).

**Western blot**

Total proteins extracted from mouse liver tissues, RAW264.7 macrophages and primary hepatocytes were assessed using the Pierce BCA protein assay kit (Thermo Fisher Scientific). The proteins were separated by SDS-PAGE (Epizyme Biotech), transferred to polyvinylidenedifluoride membranes (Bio-Rad, Hercules, CA) and incubated with the primary antibody in TBST containing 5% (wt/vol) BSA at 4 °C overnight. The blots were then incubated with the HRP-conjugated secondary antibody (1:10000, KangChen Biotech, Shanghai, China) at room temperature for 1 h. Immunoreactive bands were detected by the ECL chemiluminescence kit (Thermo Scientific Pierce, Waltham, MA). The density of the immunoblots was measured by ImageJ (National Institutes of Health) and normalized to glyceraldehyde 3-phosphate dehydrogenase (1:10000, KangChen Biotech, Shanghai, China) or β-actin (1:5000, Cell Signaling Technology). In this study, the total expression levels of TLR4, IκBα, p-IκBα, NF-κB and p-NF-κB (1:1000, all from Cell Signaling Technology) in macrophages were measured and normalized to β-actin (1:5000, Cell Signaling Technology). The total expression levels of NLRP3, IL-1β, Caspase-1, Nrf2, Keap1 and HO-1 (1:1000, all from Cell Signaling Technology) in hepatocytes were measured and normalized to GAPDH (1:10000, KangChen Biotech, Shanghai, China).
Statistical analysis

All statistical analyses were carried out with GraphPad Prism v7.03 software (GraphPad, La Jolla, CA, United States). All the data are expressed as the mean ± standard error of the mean (SEM). Statistical differences were determined by one- or two-way analysis of variance in multiple groups. Differences between two groups were analyzed by the Student’s t-test. A P value < 0.05 was considered statistically significant.

RESULTS

Lipid-laden primary hepatocytes have direct effects on M1/M2 macrophage polarization and inflammation

Because of the high plasticity and heterogeneity, macrophages can be skewed into the M1 phenotype or M2 phenotype under different microenvironments\(^{[26]}\). However, whether lipid-laden hepatocytes can affect macrophage polarization is uncertain. Here, we isolated primary hepatocytes and incubated them with FFAs. After 24h culturing, most primary hepatocytes were adhered to the plate with centered dual-nuclei and polyhedral in shape under the microscope, indicating that the primary hepatocytes were in good condition (Figure 1A) and exhibited excess lipid accumulation after FFA incubation, as shown by Oil Red O staining (Figure 1B). At the same time, the contents of TG and T-CHO generated in hepatocytes were increased significantly (Figure 1C). The mRNA expression levels of the lipid synthesis genes Fasn and Srebp1c were upregulated, and the mRNA expression levels of the lipid decomposition genes Acox1 and Cpt1a were downregulated (Figure 1D). These results suggested that the NAFLD hepatocyte model was successfully established. Next, a supernatant transfer experiment between lipid-laden hepatocytes and macrophages was established. We found that conditioned medium (CM) from FFA-incubated hepatocytes induced M1-polarized macrophages with significant upregulation of all M1 markers, including Nos2, TNFα and IL-6, and partial downregulation of M2 markers, such as IL-10 (Figure 1E). In
addition, the NF-κB signaling pathway in macrophages was also activated by CM-FFA, as demonstrated by significant increases in the protein expression levels of TLR4, p-NF-κB and p-IκBα (Figure 1F). These results demonstrated that lipid-laden hepatocytes exerted direct roles in M1 macrophage polarization and inflammation.

**Lipid-laden hepatocytes induce macrophage M1 polarization and inflammation via IL-1β signaling**

Lipid-laden hepatocytes promoted M1 macrophage polarization and inflammation; however, the possible pathways of signal exchange between the primary hepatocytes and macrophages were unclear. We found that FFA incubation obviously increased the mRNA and protein expression levels of the inflammatory factor IL-1β in hepatocytes (Figure 2A, 2B). Similarly, lipid-laden hepatocytes secreted a high level of IL-1β into the cell culture supernatant (Figure 2C). To further investigate whether IL-1β participates in the signaling passing between hepatocytes and macrophages, we pretreated macrophages with an interleukin-1 receptor antagonist (IL-1Ra) to block IL-1β receptors. Then, a supernatant transfer experiment between lipid-laden hepatocytes and macrophages was established. The results showed that IL-1β expression in macrophages was significantly decreased with IL-1Ra pretreatment (Figure 2D). As expected, we found that inhibition of IL-1β signaling with IL-1Ra significantly prevented macrophage M1 polarization induced by CM-FFAs, as shown by the downregulation of M1-type markers and the upregulation of M2-type markers (Figure 2E). Simultaneously, IL-1Ra suppressed the protein expression levels of TLR4, p-NF-κB and p-IκBα in macrophages induced by CM-FFA (Figure 2F). These results indicated that NAFLD hepatocytes induced M1 macrophage polarization and inflammatory signal activation via IL-1β signaling.

**Upregulating PPAR-γ activity ameliorates oxidative stress and NLRP3 inflammasome activation in lipid-laden hepatocytes**
PPAR-γ is a nuclear receptor that is firmly involved in lipid metabolism and the inflammatory-immune response\cite{27}. To further explore the role and properties of PPAR-γ in lipid-laden primary hepatocytes, the PPAR-γ agonist GW1929 was added to the hepatocyte culture system for 3 h before FFA incubation. The results showed that GW1929 administration significantly decreased the ROS content and IL-1β secretion level in hepatocytes treated with FFA (Figure 3A, 3B). In addition, GW1929 significantly downregulated both the mRNA and protein expression levels of NLRP3 inflammasome-related genes, including Nlrs3, Caspase-1 and IL-1β, in lipid-laden hepatocytes (Figure 3C, 3D). Furthermore, GW1929 markedly reduced the mRNA and protein expression levels of the oxidative injury marker Keap1 but enhanced the mRNA and protein expression levels of the antioxidant-related genes Nrf2 and Ho-1 (Fig. 3E, 3F). These results indicated that upregulation of PPAR-γ activity in NAFLD hepatocytes could ameliorate oxidative stress and NLRP3-IL-1β pathway activation.

**Hepatocyte-specific PPAR-γ knockout aggravates oxidative stress and NLRP3 inflammasome activation in lipid-laden hepatocytes**

To further confirm the anti-inflammatory and antioxidant effects of PPAR-γ on lipid-laden hepatocytes, we isolated primary hepatocytes from hepatocyte-specific PPAR-γ knockout mice and treated them with FFA in vitro. The mRNA expression of Ppar-γ in primary hepatocytes from hepatocyte-specific PPAR-γ knockout mice was fully knocked out (Figure 4A). As expected, the loss of PPAR-γ in hepatocytes enhanced IL-1β secretion and ROS generation after FFA incubation (Figure 4B, 4C). In addition, PPAR-γ deficiency in lipid-laden hepatocytes increased the mRNA and protein expression levels of the Nlrs3, Caspase-1 and IL-1β genes (Figure 4D, 4E). Furthermore, PPAR-γ deficiency markedly increased the mRNA and protein expression levels of Keap1 but decreased the mRNA and protein expression levels of Nrf2 and Ho-1(Figure 4F, 4G). These results further confirmed that PPAR-γ exerts a protective effect against lipid peroxidation and inflammation in lipid-laden hepatocytes.

**Regulation of PPAR-γ activity in lipid-laden hepatocytes affects macrophage polarization and inflammation**
Next, we further explored whether regulation of PPAR-γ activity in lipid-laden hepatocytes would subsequently affect macrophage polarization shifting and inflammation. Macrophages were incubated with CM derived from hepatocytes that were pretreated with GW1929 or FFA alone or with CM from PPAR-γ-deficient hepatocytes treated with FFA. Interestingly, the increased M1 marker expression was significantly downregulated and the M2 marker expression levels of Arg1 and IL-10 were markedly upregulated in macrophages incubated with CM from lipid-laden hepatocytes that were pretreated with GW1929 (CM-GW1929+FFA) compared with the CM-FFA group (Figure 5A). In addition, the protein expression levels of TLR4, p-NF-κB and p-κBα in macrophages from the CM-GW1929+FFA group were significantly decreased (Figure 5B). In contrast, depletion of PPAR-γ in lipid-laden hepatocytes significantly upregulated the mRNA expression levels of all M1 markers but decreased the mRNA expression levels of Arg1 and Mrc2 in macrophages (Figure 5C). Furthermore, depletion of PPAR-γ in lipid-laden hepatocytes further promoted activation of the TLR4/NF-κB signaling pathway in macrophages (Figure 5D). These results illustrated that regulation of PPAR-γ activity in NAFLD hepatocytes could modulate macrophage polarization and inflammation.

**Rosiglitazone improved NLRP3 inflammasome activation and oxidative stress in high-fat diet-induced NAFLD mice**

Our *in vitro* experiments demonstrated that PPAR-γ exerted anti-inflammatory and antioxidant effects in lipid-laden hepatocytes, which can further affect macrophage polarization and inflammation. Next, we further explored whether these effects also occurred in vivo. Our previous studies showed that rosiglitazone administration improved hepatic steatosis and Kupffer cell activation in high-fat diet-induced NAFLD mice. Here, we further found that rosiglitazone significantly decreased the mRNA expression levels of the Nlrp3, Caspase-1 and IL-1β genes in bulk cells from livers of high-fat diet-induced NAFLD mice (Figure 6A). Furthermore, rosiglitazone markedly downregulated the mRNA expression of Keap1 but increased the mRNA expression levels of PPAR-γ, Nrf2 and Ho-1(Figure 6B). The IL-1β level in plasma and the Caspase-
1 activity in the liver both declined after rosiglitazone administration (Figure 6C, 6D), while the SOD activity and GSH content were significantly enhanced in rosiglitazone-treated mouse plasma (Figure 6E). In contrast, oxidative injury metabolites, such as ROS and MDA, in plasma were decreased after rosiglitazone intervention (Fig. 6F). Therefore, we concluded that rosiglitazone not only alleviated hepatic steatosis and Kupffer cell activation but also improved NLRP3 inflammasome activation and oxidative stress in HF diet-fed NAFLD mice.

**DISCUSSION**

Lipid metabolism disorder is a critical initiator of the inflammatory response and immune activation in NAFLD\textsuperscript{[10]}. How lipids lead to innate immune activation is poorly understood. Here, we demonstrated that lipid-laden hepatocytes can transmit inflammatory signals to macrophages through the release of IL-1β, directly inducing M1 macrophage polarization and inflammatory activation. Regulation of PPAR-γ activity in lipid-laden hepatocytes further affected macrophage polarization and inflammation. In addition, an *in vivo* study showed that a PPAR-γ agonist improved NLRP3 inflammasome activation and oxidative stress in high-fat diet-induced NAFLD mice, expanding our understanding of the underlying mechanisms of PPAR-γ in NAFLD.

Macrophage activation is considered to be a prominent hallmark of NASH\textsuperscript{[28]}. The polarization of macrophages is firmly related to the inflammatory state. Previous studies have suggested that macrophages are more likely to transform from the M1 to the M2 phenotype if activation of the NF-κB signaling pathway is inhibited\textsuperscript{[15]}. TLR4 deficiency directly alters the polarization of adipose tissue macrophages toward alternative activation\textsuperscript{[20]}. Upon activation, macrophages not only release inflammatory cytokines and chemokines but also regulate the status or function of surrounding cells\textsuperscript{[13, 30]}. Recent studies have demonstrated that selective depletion of Kupffer cells reduced liver steatosis and monocyte infiltration in NASH\textsuperscript{[31-33]}. Our previous study also confirmed that macrophages/Kupffer cells polarized by fatty acids can regulate lipid metabolism in hepatocytes\textsuperscript{[20]}. This result suggested that there appears to be a potential
interaction between macrophages and hepatocytes in NAFLD. In the current study, our results revealed that lipid-laden hepatocytes directly induced macrophage M1 polarization and TLR4/NF-κB pathway activation. Through an in-depth study, we found that IL-1β secreted by lipid-laden hepatocytes mediates the communication between hepatocytes and macrophages. Interestingly, we confirmed that when macrophages were blocked by an IL-1β receptor inhibitor, M1 macrophage polarization induced by lipid-laden hepatocytes was weakened. Furthermore, we proved that residual fatty acids in hepatocyte supernatant alone were not sufficient to induce macrophages toward M1 polarization (Figure S1).

PPAR-γ is a ligand-activated nuclear receptor with potent anti-inflammatory properties that is involved in the regulation of immune and inflammatory responses. A recent study demonstrated that modulation of PPAR-γ activity attenuated HFD-induced NAFLD by regulating lipid metabolism and oxidative stress in hepatocytes via Nrf2 activation. Due to the lower expression of PPAR-γ in hepatocytes, the role of PPAR-γ in hepatocytes is not fully understood. In the current study, we found that ROS generation and IL-1β secretion in lipid-laden hepatocytes were significantly reduced when cells were pretreated with the PPAR-γ agonist GW1929. Recently, a study reported that PPAR-γ exerted an anti-inflammatory role by suppressing NLRP3 inflammasome activation in macrophages. Here, we revealed that a PPAR-γ agonist exerted an anti-inflammatory effect by inhibiting NLRP3 inflammasome activation in lipid-laden hepatocytes. In contrast, PPAR-γ deficiency in hepatocytes enhanced ROS generation, NLRP3 inflammasome activation, and IL-1β secretion after FFA treatment in vitro.

Interestingly, we further found that the upregulation of PPAR-γ activity in lipid-laden hepatocytes subsequently reversed macrophage M1 polarization and reduced activation of the TLR4/NF-κB pathway. Conversely, PPAR-γ depletion in lipid-laden hepatocytes exacerbated macrophage M1 polarization and TLR4/NF-κB pathway activation. This result suggested that the regulation of PPAR-γ activity in hepatocytes played an important role in the interaction between lipid-laden hepatocytes and
macrophages. Our previous study revealed that rosiglitazone, a PPAR-γ agonist, significantly alleviated hepatic lipid deposition and Kupffer cell activation in HFD-induced NAFLD mice\textsuperscript{[17]}. In the present study, our results also clearly demonstrated that rosiglitazone mitigated NLRP3 inflammasome activation and oxidative stress in HFD-induced NAFLD mice.

A recent study showed that hepatocyte-specific PPAR-γ disruption reduced hepatic steatosis but increased hepatic neutrophil infiltration after HFD-plus-binge ethanol\textsuperscript{[38]}. PPAR-γ deletion in hepatocytes highly augmented PA- or TNF-α-induced production of Cxcl1. This result indicated that PPAR-γ activation in hepatocytes exerted an anti-inflammatory effect, which was consistent with our findings. Another study found that hepatocyte-specific loss of PPAR-γ reduced the progression of high fat, cholesterol, and fructose (HFCF)-induced NASH in mice\textsuperscript{[39]}. These findings seemed to suggest that hepatocyte PPAR-γ contributed to the development of inflammation and fibrosis in NASH. However, the hepatocyte-specific loss of PPAR-γ did not reduce hepatic steatosis in HFCF- or MCD-induced NASH\textsuperscript{[39]}. These contradictory experimental results may be due to the use of different animal models in the studies. In the current study, lipid-laden hepatocytes were established by excess FFA incubation in vitro. The classical lipogenic property of PPAR-γ is significantly involved in this condition\textsuperscript{[40]}. In addition, we further found that PPAR-γ activation exerted obvious antioxidant and anti-inflammatory effects in lipid-laden hepatocytes. We assumed that FFA overload directly led to lipotoxicity on hepatocytes, which contributed to lipid peroxidation. Thus, PPAR-γ was upregulated and exerted antioxidant effects against ROS to mitigate cell injury and downstream inflammatory events. Therefore, the role of PPAR-γ in hepatocytes is firmly related to different models, diet patterns and cellular stimulations. PPAR-γ is a complex nuclear receptor with specific dominant properties in various disease courses.

**CONCLUSION**
In conclusion, our study revealed that lipid-laden hepatocytes significantly skewed macrophage polarization to M1 phenotype. Up-regulation of PPAR-γ activity in lipid-laden hepatocytes improved macrophage M1 polarization and inflammation by attenuating hepatocytes oxidative stress and NLRP3 inflammasome activation. Strategies that target regulation of PPAR-γ activity to modulate cells cross-talk will be beneficial for treating NAFLD.

**ARTICLE HIGHLIGHTS**

**Research background**
Lipid metabolism disorder and inflammatory-immune activation are two vital triggers in NAFLD. Little is known about regulation of PPAR-γ activity in modulating cells cross-talk in NAFLD.

**Research motivation**
It remains unknown the role of PPAR-γ in hepatocytes as well as in the interaction between hepatocytes and macrophages in NAFLD.

**Research objectives**
To investigate whether the regulation of PPAR-γ activity in lipid-laden hepatocytes affects macrophage polarization and inflammation and explore the potential mechanisms.

**Research methods**
Primary hepatocytes were isolated from wild-type C57BL/6J mice or hepatocyte-specific PPAR-γ knockout mice and incubated with free fatty acids (FFAs). Macrophages were incubated with conditioned medium (CM) from lipid-laden hepatocytes with or without the PPAR-γ agonist. Wild-type C57BL/6J mice were fed a high-fat diet and administered rosiglitazone.
Research results
Primary hepatocytes exhibited significant lipid deposition and increased ROS production after FFA incubation. Conditioned medium from lipid-laden hepatocytes promoted macrophage polarization to the M1 type and activation of the TLR4/NF-κB pathway. A PPAR-γ agonist ameliorated oxidative stress and NLRP3 inflammasome activation in lipid-laden hepatocytes and subsequently prevented M1 macrophage polarization. Hepatocyte-specific PPAR-γ deficiency aggravated oxidative stress and NLRP3 inflammasome activation in lipid-laden hepatocytes, which further promoted M1 macrophage polarization. Rosiglitazone administration improved oxidative stress and NLRP3 inflammasome activation in HF diet-induced NAFLD mice in vivo.

Research conclusions
Up-regulation of PPAR-γ activity alleviated NAFLD through modulation of the crosstalk between hepatocytes and macrophages by the ROS-NLRP3-IL-1β signaling pathway.

Research perspectives
To elaborate the underlying pathogenesis of NAFLD from the perspective of inflammation and immune activation.
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