BMC Biology

RESEARCH

Open Access

PPARα regulates YAP protein levels and activity by affecting its ubiquitination modification

Wenhong Zhou[1,3], Shuaishuai Zhang[1], Yao Chen[1], Ziqi Chen[1], Guofang Bi[1], Manlan Guo[1], Xiaowen Jiang[1], Xiao Yang[1,2], Jianhong Fang[1], Linhu Ye[3*], Shicheng Fan[1*] and Huichang Bi[1,2*]

Abstract

Background Peroxisome proliferator-activated receptor α (PPARα) plays a crucial role in liver physiological and pathological processes. Yes-associated protein (YAP) is a key effector in regulating cell growth and organ size. Ubiquitination is known to modulate YAP protein expression, stability, and nuclear localization. Our previous study demonstrated that PPARα activation promotes hepatomegaly and liver regeneration via YAP activation. However, the underlying molecular mechanisms by which PPARα regulates YAP are unclear. In this study, PPARα was activated by the classical agonist WY-14643, and its effects on YAP ubiquitination were examined using plasmid transfection and immunoprecipitation. The ubiquitination of YAP was further investigated through mutant YAP plasmids, gene knockdown, and immunofluorescence staining. YAP mRNA and protein expression were measured via qRT-PCR and western blotting.

Results The results demonstrated that PPARα activation upregulated YAP protein levels and enhanced its activity, while reducing overall YAP ubiquitination. Specifically, PPARα activation inhibited K48-linked ubiquitination while promoting K63-linked ubiquitination of YAP. Mutations at the K252, K321, and K497 residues of YAP markedly reduced the capacity of PPARα activation to facilitate YAP nuclear translocation. Furthermore, knockdown of the E3 ligase *TRAF6* abolished the PPARα-induced K63-linked ubiquitination of YAP and the upregulation of its downstream target genes.

Conclusions These findings highlight the pivotal role of ubiquitination in regulating YAP through PPARα activation, providing novel insights for future studies on the post-translational regulation of YAP by PPARα activation.

Keywords Peroxisome proliferator-activated receptor α, Yes-associated protein, Ubiquitination, TRAF6

*Correspondence:
Linhu Ye
yelinhu@126.com
Shicheng Fan
fanshch3@smu.edu.cn
Huichang Bi
bihchang@smu.edu.cn
[1] NMPA Key Laboratory for Research and Evaluation of Drug Metabolism & Guangdong Provincial Key Laboratory of New Drug Screening & Guangdong-Hongkong-Macao Joint Laboratory for New Drug Screening, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, China
[2] The State Key Laboratory of Chemical Oncogenomics, School of Chemical Biology and Biotechnology, Shenzhen Graduate School of Peking University, Shenzhen 518055, China
[3] Department of Pharmacy, The Sixth Affiliated Hospital, School of Medicine, South China University of Technology, Foshan 528200, China

Background

Peroxisome proliferator-activated receptor α (PPARα, NR1C1) is an important member of the nuclear receptor superfamily that plays a pivotal role in the regulation of lipid metabolism, energy homeostasis, and inflammation [1, 2]. PPARα is highly expressed in tissues with active fatty acid catabolism, such as the liver, heart, and kidney [3]. It classically acts as a transcription factor, binding to peroxisome proliferator response elements (PPREs) within the promoters of target genes to regulate their expression [4]. PPARα is a ligand-activated transcription factor, whose agonists can be classified as either endobiotics or xenobiotics [5]. WY-14643, fenofibrate, and gemfibrozil are known as exogenous PPARα agonists for their ability to bind to and activate PPARα, among which WY-14643 exerts the highly potent and selective activation [6]. The administration of WY-14643 has been shown to promote hepatocyte proliferation, leading to significant hepatic enlargement [7]. Due to its critical role in metabolic regulation, PPARα also plays a key role in various hepatic physiological and pathological processes [8].

Yes-associated protein (YAP) is the crucial component of the Hippo signaling pathway and plays an important role in the control of cell proliferation and organ size [9]. As a transcriptional coactivator, YAP exerts its biological function through nuclear translocation [10]. The upstream regulators of the Hippo pathway ultimately dictate YAP's localization, determining whether it translocates into the nucleus [11]. When YAP is localized in the nucleus, it interacts with TEAD family transcription factors to activate a series of target genes, thereby participating in the regulation of cell fate [12].

Our previous studies have demonstrated that the activation of PPARα via WY-14643 significantly promotes liver enlargement and regeneration in mice by activating and interacting with the YAP signaling pathway. WY-14643 treatment induces YAP translocation from cytoplasm to nucleus [13]. Similar activation of the YAP signaling pathway was also observed during fenofibrate-induced hepatomegaly and liver regeneration in mice [14]. However, the regulatory mechanism of PPARα activation on YAP activity remains unclear.

Ubiquitination is an important mechanism to regulate protein expression and signal transduction. Polyubiquitination chains are covalently linked to target proteins through specific ubiquitin ligases, with different linkages producing distinct functional outcomes [15]. A large number of studies have shown that the expression and activity of YAP are strictly controlled by ubiquitination modification [16]. For instance, HECT domain and RCC1-like domain-containing protein 3 (HERC3) stabilizes YAP/TAZ expression and prevents its degradation by blocks β-TrCP-mediated ubiquitination [17]. IL-1β activates TNF receptor-associated factor 6 (TRAF6) to promote the K63-linked ubiquitination of YAP at the K252 site, leading to enhanced stability and nuclear localization of YAP by interfering with its binding to proteins containing PPXY motifs such as large tumor suppressor 1 (LATS1) and angiomotin (AMOT) [18]. Ubiquitin-specific peptidase 36 (USP36) stabilizes the YAP protein by inhibiting the K48-linked polyubiquitination of YAP, thereby promoting the progression of esophageal squamous cell carcinoma [19]. By ubiquitinating or deubiquitinating YAP at different sites, the stability and activity of YAP can be modulated.

Considering the role of ubiquitination in the regulation of YAP, it is interesting to investigate whether PPARα activation modulates YAP ubiquitination. This study aims to investigate the role of PPARα in regulating YAP ubiquitination and to elucidate the underlying molecular mechanisms, providing novel insights into the interaction between PPARα and YAP.

Results

PPARα activation enhances YAP protein levels and activity

To investigate the effect of PPARα activation on YAP, HepG2 cells were treated with the PPARα classical agonist WY-14643. WY-14643 treatment significantly upregulated the expression of PPARα downstream target protein Acyl CoA oxidase 1 (ACOX1) (Fig. 1A–B), indicating the activation of PPARα. Consistent with our previous findings [13], the protein expression of YAP was upregulated while the protein expression of phosphorylated YAP (p-YAP) was downregulated after WY-14643 treatment (Fig. 1A–B). Meanwhile, RT-qPCR results

(See figure on next page.)

Fig. 1 PPARα activation enhances YAP protein levels and activity. **A** HepG2 cells were treated with 20 μM WY-14643 and the expression of YAP, p-YAP, and ACOX1 protein were detected by western blot. **B** The relative protein expression of YAP, p-YAP, and ACOX1. **C** HepG2 cells were treated with WY-14643 and the expression of *ACOX1*, *YAP*, and its downstream target genes were detected by RT-qPCR. **D** HepG2 cells were treated with WY-14643 and the expression of LATS1, p-LATS1/2, MST1, MST2 and p-MST1/2 protein were detected by western blot. **E** The relative protein expression of LATS1, p-LATS1/2, MST1, MST2 and p-MST1/2. **F** HepG2 cells were treated with MG132 (20 μM) and the expression of YAP protein was detected by western blot. **G** The relative protein expression of YAP. **H** The Vehicle group and the WY-14643 group cells were treated with CHX (50 mg/mL) and the expression of YAP protein was detected by western blot. **I** The relative protein expression of YAP. Data are presented as the mean ± S.D. * $P < 0.05$, ** $P < 0.01$, ns, not significant, compared with the Vehicle group

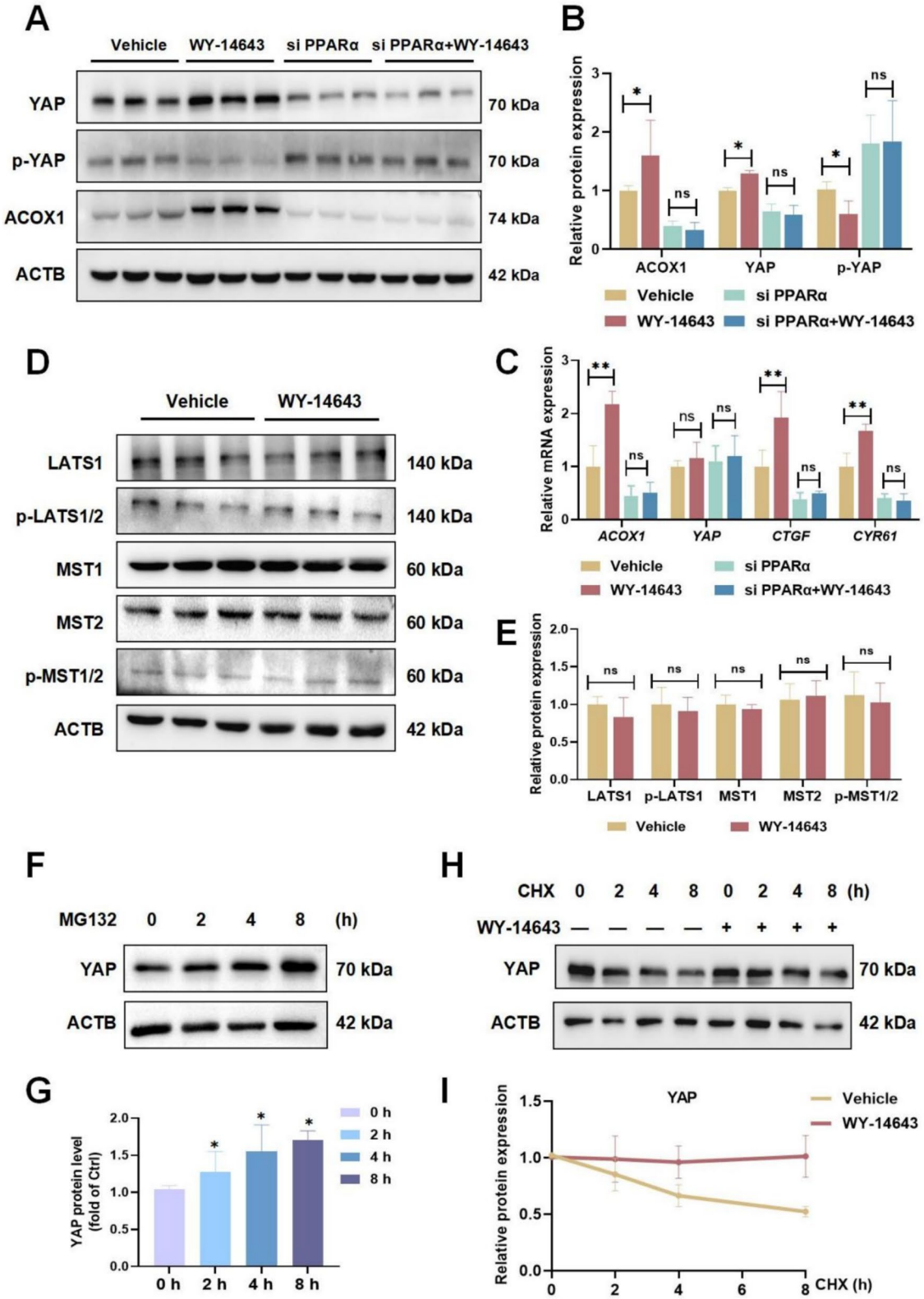

Fig. 1 (See legend on previous page.)

showed that both *ACOX1* and the YAP downstream target genes, cysteine-rich angiogenic inducer 61 (*CYR61*) and connective tissue growth factor (*CTGF*), were significantly upregulated after WY-14643 treatment (Fig. 1C), indicating that PPARα activation enhanced both YAP protein levels and activity. Furthermore, we utilized siRNA to knock down *PPARα* expression, and the knockdown efficiency of *PPARα* was verified by RT-qPCR (Additional file 1: Fig. S1A). The knockdown of *PPARα* markedly abrogated the WY-14643-upregulated expression of YAP protein and its target genes (Fig. 1A–C). Notably, the mRNA level of *YAP* did not show significant change following PPARα activation (Fig. 1C), suggesting that PPARα activation may regulate YAP at the translational rather than the transcriptional level.

The phosphorylation of YAP, mediated by Hippo pathway, is a well-established mechanism for regulating YAP activity [20]. We further assessed the expression of upstream kinases in the Hippo pathway. The results showed that the expression of MST1/2, LATS1/2, and their phosphorylated forms, p-MST1/2 and p-LATS1/2, were not significantly changed upon PPARα activation (Fig. 1D–E). These findings suggest that PPARα activation may not regulate YAP phosphorylation via the upstream phosphokinase of the Hippo pathway.

We further investigated whether PPARα activation impacts the stability of the YAP protein. HepG2 cells were treated with the proteasome inhibitor MG132 for 0, 2, 4, and 8 h. The results showed that the YAP protein level was increased with prolonged treatment (Fig. 1F–G), suggesting that YAP was degraded through the proteasome pathway in HepG2 cells. Then we utilized cycloheximide (CHX), a well-established inhibitor of protein biosynthesis [21], to assess the effects of PPARα activation on YAP protein stability. The results showed that YAP protein degradation was significantly slower in the WY-14643 group compared to the Vehicle group (Fig. 1H–I), indicating that PPARα activation may enhance YAP protein stability by regulating its degradation. Collectively, these findings demonstrated that PPARα activation increased both the levels and activity of YAP protein, underscoring its regulatory role in YAP stability and function.

PPARα activation regulates YAP ubiquitination modification

To investigate the effect of PPARα activation on the ubiquitination of YAP, plasmid transfection, and Co-IP experiments were performed. The findings revealed that WY-14643 treatment inhibited the overall ubiquitination of YAP in HepG2 cells (Fig. 2A). We further examined the effects of PPARα activation on K63-linked and K48-linked ubiquitination, two types of YAP ubiquitination modifications. Upon WY-14643 treatment, the K48-linked ubiquitination of YAP was reduced (Fig. 2B), whereas the K63-linked ubiquitination of YAP was increased (Fig. 2C). Fenofibrate, another well-established PPARα agonist, was used to investigate the effect of PPARα activation on ubiquitination of YAP, and consistent results were observed (Additional file 1: Fig. S2A-C). Moreover, *PPARα* knockdown significantly enhanced the ubiquitination of YAP, and WY-14643 treatment failed to suppress YAP ubiquitination in *PPARα*-knockdown cells (Fig. 2D). In conclusion, WY-14643 treatment modulated the ubiquitination of YAP in a PPARα-dependent manner.

The K63-linked ubiquitination mediated by PPARα activation is associated with YAP nuclear localization

Previous studies have indicated that YAP nuclear localization is influenced by K63-linked ubiquitination at lysine residues 252, 321, and 497 [18, 22]. To further explore how PPARα activation induces YAP nuclear translocation, we investigated the association between YAP nuclear localization and its K63-linked ubiquitination following PPARα activation. We constructed several YAP mutants to replace lysine residues at site 252 (K252R), 321 (K321R), and 497 (K497R) with arginine (Fig. 3A). The K63-linked ubiquitination of these mutants was assessed in HepG2 cells after WY-14643 treatment. Compared to cells transfected with wild-type YAP, K63-linked ubiquitination in the K252R, K321R, and K497R mutants was not enhanced by WY-14643 treatment (Fig. 3B). These results suggest that lysine residues 252, 321, and 497 are critical for promoting K63-linked ubiquitination of YAP following PPARα activation. To detect the nuclear localization of YAP, immunofluorescence staining was conducted. The nuclear-to-total fluorescence intensity ratio of YAP was used to quantify the effect of PPARα activation on YAP nuclear translocation. There was a significant increase in the nuclear distribution of wild-type YAP and the YAP mutants K252R, K321R, and K497R after PPARα activation, but the YAP mutants K252R, K321R, and K497R showed a markedly reduced nuclear localization compared to wild-type YAP after WY-14643 treatment (Fig. 3C–D), indicating that mutation of the K63-linked ubiquitination sites impairs the ability of YAP to undergo nuclear translocation following PPARα activation. In summary, PPARα activation may regulate YAP nuclear localization by modulating its K63-linked ubiquitination.

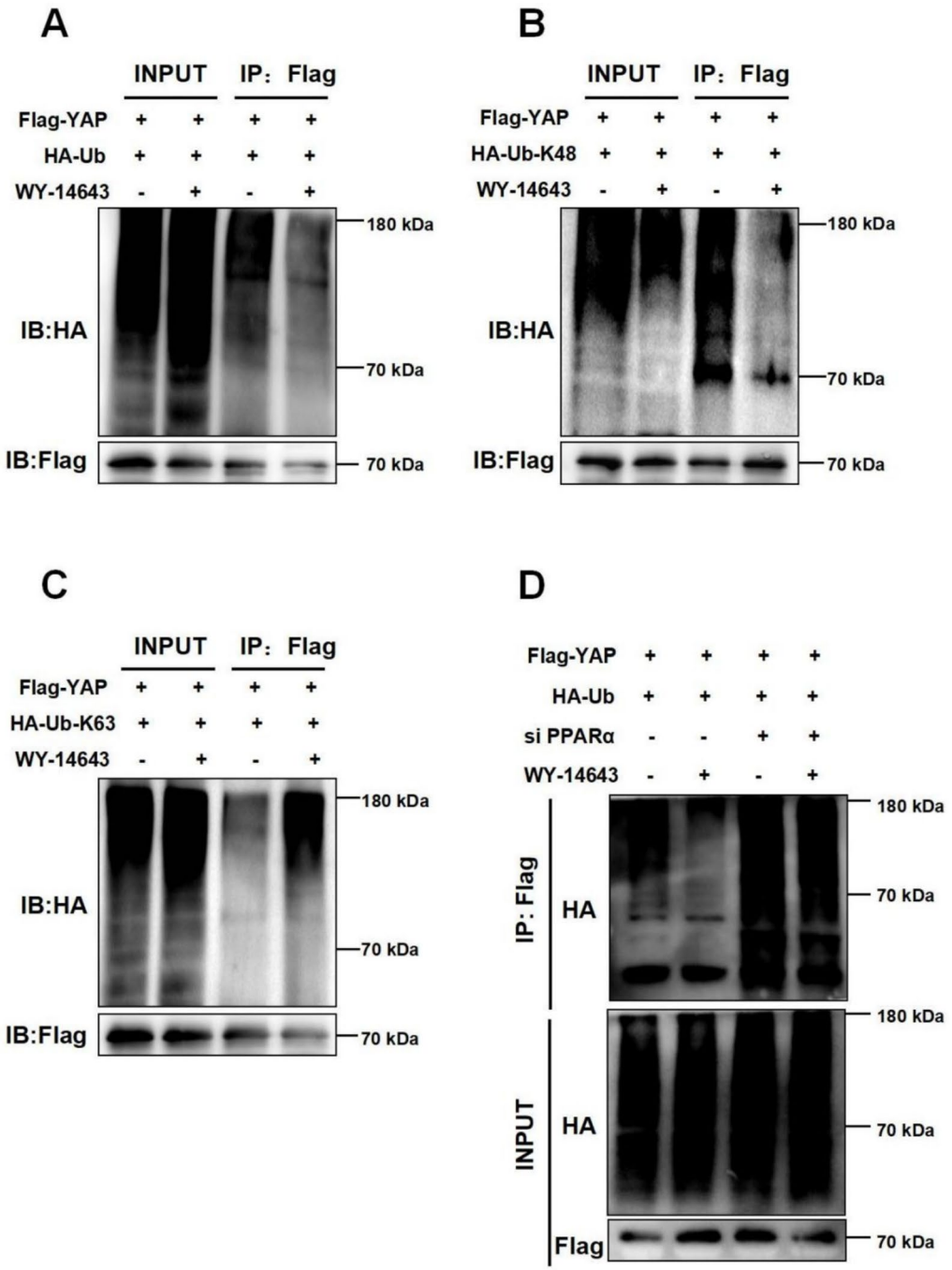

Fig. 2 PPARα activation regulates YAP ubiquitination. **A-C** HepG2 cells were co-transfected with Flag-YAP and HA-tagged ubiquitin (HA-Ub) plasmids (**A**)/mutant HA-Ub-K48 plasmids (**B**)/mutant HA-Ub-K63 plasmids (**C**) and then treated with 20 μM WY-14643. Before harvesting, all cells were treated with 20 μM MG132 for 6 h. YAP protein was immunoprecipitated using an anti-FLAG antibody, and ubiquitinated YAP proteins were detected using an anti-HA antibody. **D** *PPARα*-knockdown and control cells were co-transfected with Flag-YAP and HA-Ub plasmids, followed by treatment with 20 μM WY-14643. Before harvesting, all cells were treated with 20 μM MG132 for 6 h. YAP protein was immunoprecipitated using an anti-FLAG antibody, and ubiquitinated YAP proteins were detected using an anti-HA antibody

PPARα activation regulates K63-linked ubiquitination of YAP via TRAF6

Tumor necrosis factor receptor-associated factor 6 (TRAF6), an E3 ligase, promotes the nuclear localization and activity of YAP by binding to it and regulating its K63 ubiquitination [18, 23]. To determine whether

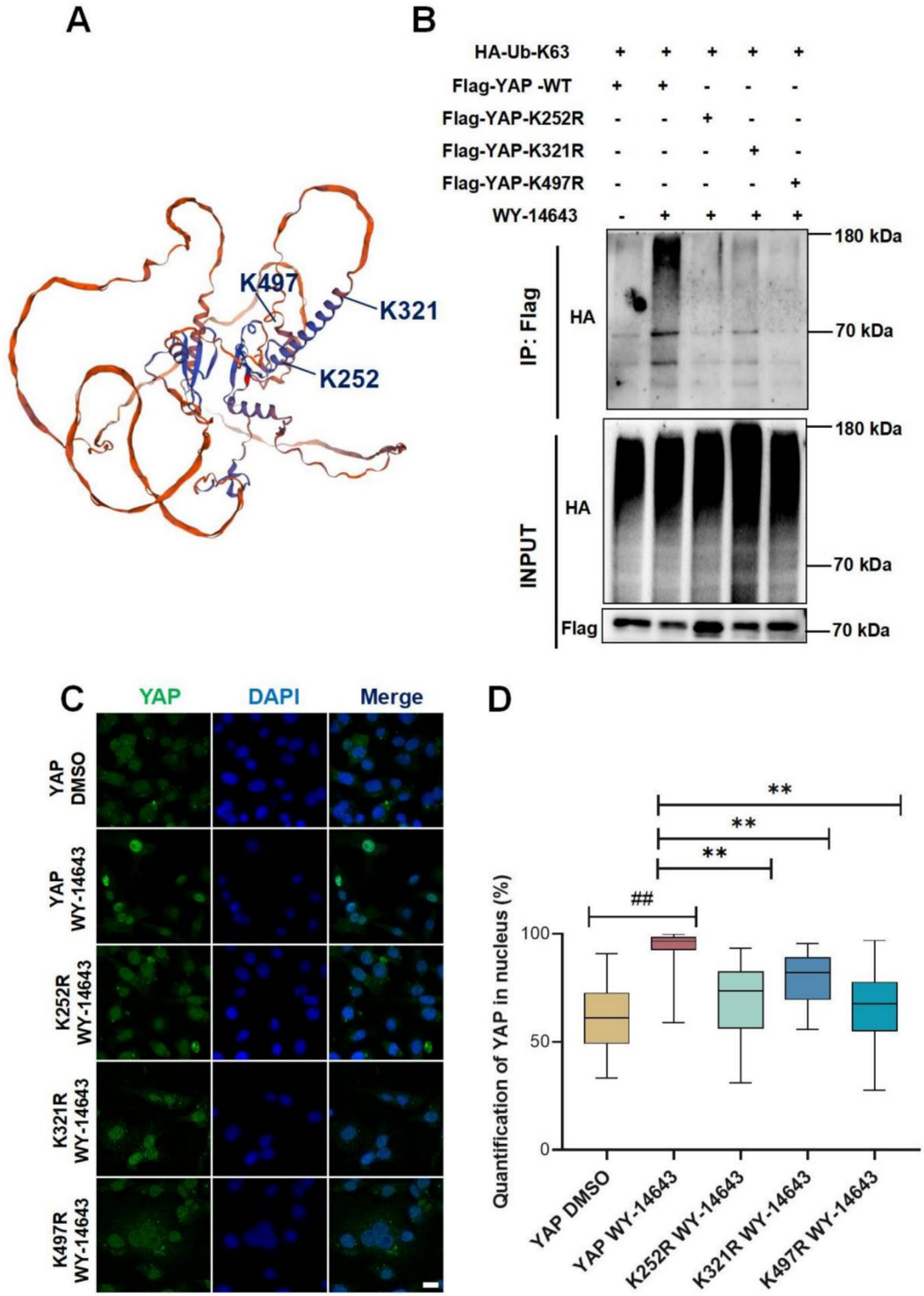

Fig. 3 K63-linked ubiquitination mediated by PPARα activation is associated with YAP nuclear localization. **A** Three-dimensional structure diagram of YAP protein. **B** HepG2 cells were co-transfected with HA-Ub-K63, Flag-YAP, or mutant of Flag-YAP plasmids, followed by treatment with 20 μM WY-14643. Before harvesting, all cells were treated with 20 μM MG132 for 6 h. YAP protein was immunoprecipitated using an anti-FLAG antibody, and ubiquitinated YAP proteins were detected using an anti-HA antibody. **C** HepG2 cells were transfected with Flag-YAP (wild-type or K252R/K321R/K497R) and then treated with 20 μM WY-14643 24 h. Immunostaining was performed using a YAP-specific antibody (green), with DAPI (blue) used for DNA staining. Scale bar = 20 μm. **D** Quantification of immunofluorescence data. ## $P < 0.01$, the WY-14643 group vs. the Vehicle group; ## **$P < 0.01$, the mutant Flag-YAP group vs. the Flag-YAP-WT group

TRAF6 plays a key role in the regulation of K63-linked ubiquitination of YAP after PPARα activation, we constructed a *TRAF6* knockdown cell model by transient transfection of *TRAF6* siRNA in HepG2 cells. The knockdown efficiency of *TRAF6* was verified by RT-qPCR (Additional file 1: Fig. S3A). Notably, treatment with the PPARα agonist WY-14643 significantly increased YAP protein levels while reducing p-YAP protein levels. However, these effects were abolished in *TRAF6*-knockdown cells (Fig. 4A–B). Inhibition of *TRAF6* almost completely abolished PPARα activation-induced K63-linked ubiquitination of YAP (Fig. 4C) and suppressed the upregulation of YAP downstream target genes *CTGF* and *CYR61* (Fig. 4D). Although previous studies have suggested that S-phase kinase-associated protein 2 (SKP2) also regulates K63-linked ubiquitination of YAP [22], these results indicated that *SKP2* knockdown did not significantly impact the K63-linked ubiquitination of YAP following PPARα activation (Additional file 1: Fig. S3C). The knockdown efficiency of *SKP2* was confirmed by RT-qPCR, as shown in Fig. S3B. These findings indicate that TRAF6, but not SKP2, mediates PPARα activation-induced K63-linked ubiquitination of YAP.

Previous studies showed that TRAF6 interacted with the WW domain of YAP to regulate its ubiquitination [18], and we also observed that PPARα interacted with the WW domain of YAP (Additional file 1: Fig. S4). To determine whether PPARα activation affected the interaction between TRAF6 and YAP, we conducted a Co-IP experiment between TRAF6 and YAP. The results showed that PPARα activation did not enhance the interaction between TRAF6 and YAP (Fig. 4E), and the mechanism by which PPARα regulates YAP via TRAF6 required further elucidation. Overall, these results suggest that TRAF6 plays an important role in the regulation of K63-linked ubiquitination of YAP by PPARα activation.

Discussion

Our previous study demonstrated that PPARα enhances the nuclear translocation and downstream target gene expression of YAP through its interaction with YAP, thereby promoting liver enlargement and regeneration in mice [13]. However, the precise mechanism by which PPARα regulates YAP activity remains unclear. In this study, we found that treatment with the PPARα agonist WY-14643 enhanced YAP protein levels and activity. Mechanistically, we revealed that PPARα activation inhibited K48-linked ubiquitination of YAP and promoted K63-linked ubiquitination of YAP. PPARα activation mediated K63-linked ubiquitination of YAP at K252, K321, and K497. The mutation of the K63-linked ubiquitination sites inhibited PPARα-induced YAP nuclear translocation. Furthermore, PPARα activation regulated K63-linked ubiquitination in a TRAF6-dependent manner. These findings demonstrate that PPARα activation leads to YAP activation through the regulation of TRAF6-driven K63-linked YAP ubiquitination (Fig. 5).

Post-translational modifications are critical for regulating YAP activity, with phosphorylation being one of the key regulatory mechanisms [24]. When the Hippo signaling pathway is activated, YAP can be phosphorylated at five serine residues (Ser61, Ser109, Ser127, Ser164, and Ser397) by LATS1/2 [10]. Among these, phosphorylation of YAP at Ser127 promotes its binding with cytoplasmic 14-3-3 proteins, preventing YAP from translocating into the nucleus [25]. In this study, we found that PPARα activation inhibited the phosphorylation of YAP at Ser127. YAP phosphorylation is primarily regulated by the upstream MST1/2 and LATS1/2 kinases [26]. Therefore, we further examined the protein expression levels of upstream kinases in the Hippo pathway following PPARα activation. However, PPARα activation did not significantly affect the expression of these upstream kinases. Further studies are needed to investigate how PPARα activation regulates YAP phosphorylation. In addition to phosphorylation, ubiquitination also plays a significant role in the regulation of YAP activity [27]. Different types of ubiquitin modifications result in distinct outcomes for YAP protein. Generally, K48-linked ubiquitination targets YAP for degradation via the 26S proteasome, thereby reducing its protein levels [28]. Conversely, K63-linked ubiquitination has been shown to enhance nuclear localization and transcriptional activity of YAP [29]. Our current study showed that PPARα activation significantly inhibited K48-linked ubiquitination of YAP while promoting K63-linked ubiquitination of YAP, indicating that PPARα activation may regulate YAP activity by modulating the balance between two types of YAP ubiquitination, ultimately leading to the activation of YAP signaling.

Various ubiquitin ligases regulate YAP ubiquitination at different sites, thereby influencing its activity [30]. Given that K63-linked ubiquitination of YAP has been demonstrated to promote its nuclear translocation and enhance its activity [29], we further examined the role of K63-linked ubiquitination in augmenting YAP protein levels and activity following PPARα activation. Our findings revealed that PPARα activation mediated K63-linked ubiquitination of YAP at multiple lysine residues, including K252, K321, and K497. Mutation of these sites significantly inhibited PPARα-induced K63-linked ubiquitination of YAP and reduced its nuclear translocation. These results suggest that PPARα activation may regulate YAP nuclear localization through K63-linked ubiquitination. It has been reported that the E3 ligase

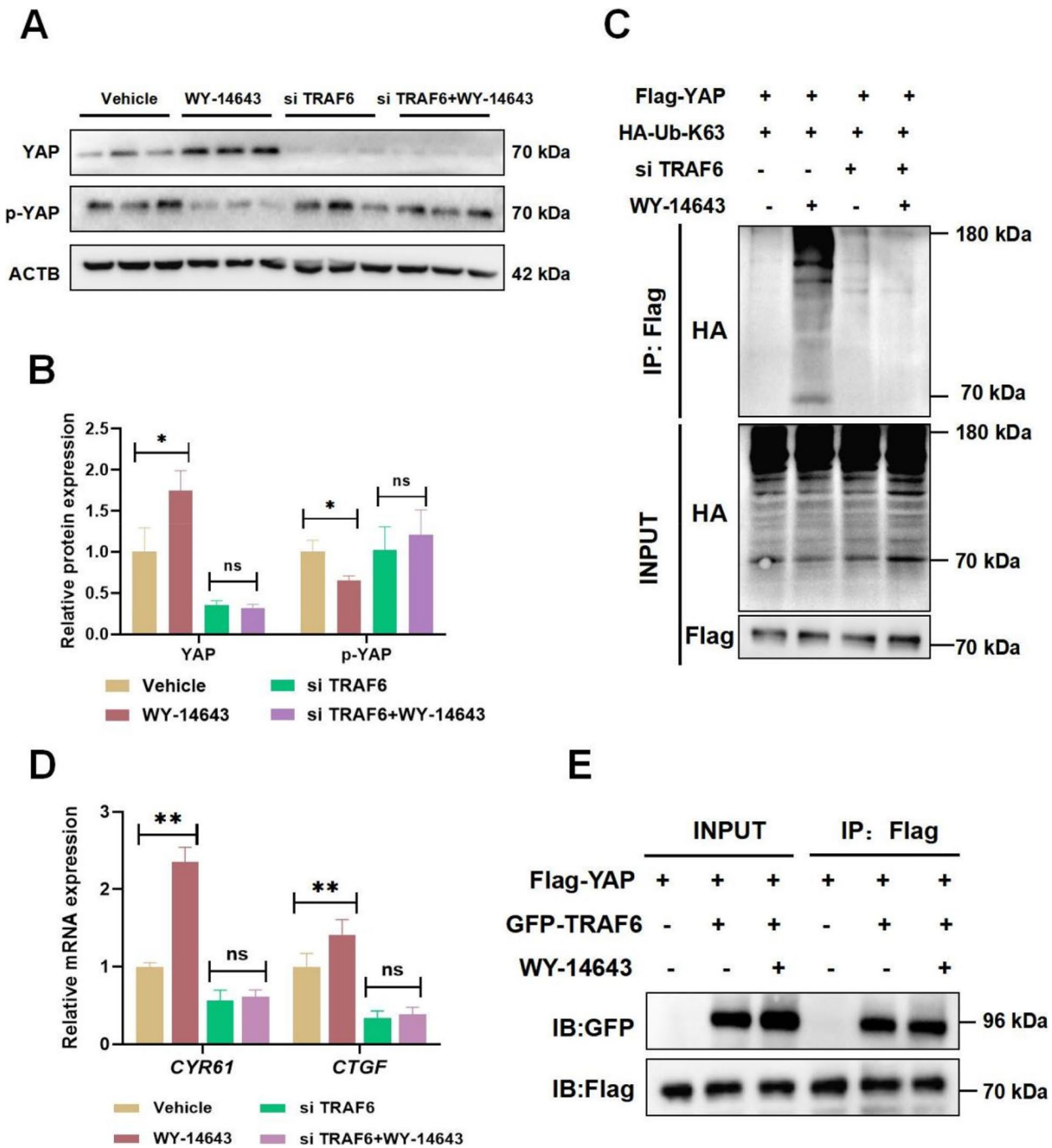

Fig. 4 PPARα activation regulates the K63-linked ubiquitination of YAP via TRAF6. **A** The expression of YAP and p-YAP protein were detected by western blot. **B** The relative protein expression of YAP and p-YAP. **C** *TRAF6*-knockdown and control cells were co-transfected with Flag-YAP and HA-Ub-K63 plasmids, followed by treatment with 20 μM WY-14643. All cells were treated with 20 μM MG132 for 6 h before harvesting. The YAP protein was immunoprecipitated using an anti-FLAG antibody and the ubiquitinated YAP proteins were analyzed using anti-HA antibody. **D** The expression of YAP downstream target genes was detected by RT-qPCR. *$P < 0.05$; **$P < 0.01$, the WY-14643 group vs. the Vehicle group. **E** HepG2 cells were cotransfected with Flag-YAP and GFP-TRAF6 plasmids and then treated with 20 μM WY-14643. The YAP protein was pulled down using an anti-FLAG antibody and the TRAF6 protein was analyzed using an anti-GFP antibody

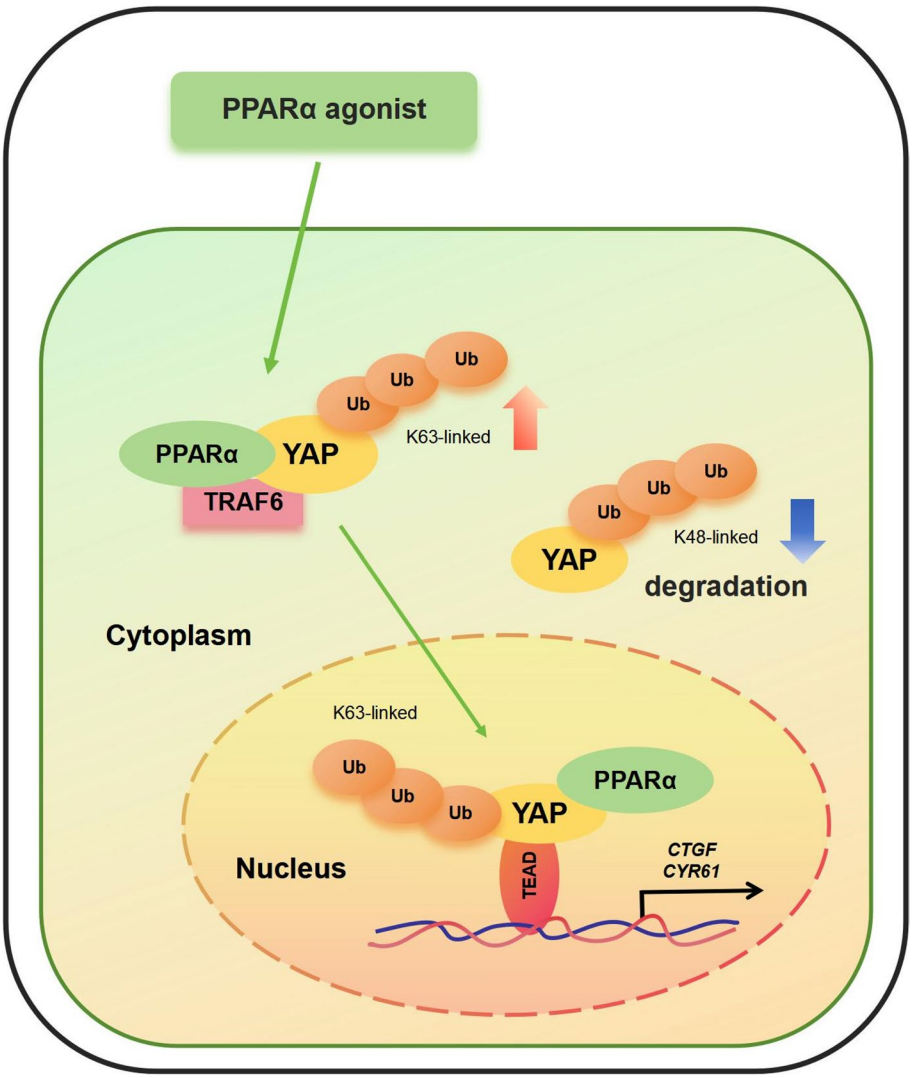

Fig. 5 Mechanistic diagram of K63-linked ubiquitination of YAP after PPARα activation

TRAF6 mediates YAP ubiquitination at K252, disrupting its interaction with angiomotin and promoting YAP nuclear translocation, highlighting the role of TRAF6 in regulating YAP activation via ubiquitination [18]. We therefore further investigated the role of TRAF6 in PPARα-mediated YAP ubiquitination. Interestingly, knockdown of *TRAF6* mRNA significantly diminished PPARα activation-induced K63-linked ubiquitination of YAP, as well as the upregulation of YAP target genes, *CTGF* and *CYR61*. This suggests that PPARα activation may enhance K63-linked ubiquitination of YAP through modulation of TRAF6. Although SKP2, another E3 ligase, has also been reported to facilitate K63-linked ubiquitination of YAP [22], our findings indicated that *SKP2* knockdown did not significantly affect K63-linked ubiquitination of YAP following PPARα activation, suggesting that SKP2 is not a major regulator of this modification in this context. Therefore, we hypothesize that other E3 ubiquitin ligases, beyond TRAF6, may contribute to the regulation of K63-linked ubiquitination at these specific residues in response to PPARα activation. However, further studies are needed to identify these potential regulators.

Next, we aimed to explore how PPARα influences YAP through TRAF6. Previous studies have reported that TRAF6 interacts with the WW domain of YAP, regulating its ubiquitination. Our results also showed that PPARα interacted with the WW domain of YAP, leading us to hypothesize that PPARα activation might also affect the interaction between TRAF6 and YAP. However, PPARα activation did not enhance the interaction between TRAF6 and YAP. Future studies should

investigate the effects of PPARα activation on TRAF6 protein expression and enzyme activity to clarify the specific role of TRAF6 in YAP regulation following PPARα activation.

Several issues still require further investigation. While we have demonstrated the mechanism of PPARα-regulated YAP ubiquitination in cell models, further validation in animal models is essential to confirm its relevance in physiological and pathological processes, such as liver enlargement and regeneration. Additionally, YAP undergoes various other post-translational modifications, including acetylation, SUMOylation, and methylation [10]. We previously reported that another nuclear receptor, PXR, regulated YAP activity by modulating its post-translational modifications, including Sirt2-dependent deacetylation and K63-linked ubiquitination, while inhibiting K48-linked ubiquitination [31]. Future research should investigate the role of posttranslational modifications in the regulation of YAP by PPARα activation, as well as whether these modifications interact temporally or spatially.

Conclusions

In conclusion, this study uncovers a potential mechanistic link between PPARα activation and the regulation of YAP through post-translational modifications. PPARα activation significantly modulates YAP ubiquitination, thereby influencing its expression and activity. These findings provide new insights into the regulation of YAP following PPARα activation and highlight TRAF6 as a potential target among ubiquitination regulators for future investigation.

Methods

Cell culture

The HepG2 cells were purchased from the American Type Culture Collection (ATCC). For routine culture, the cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotics (penicillin–streptomycin) and incubated in a cell incubator at 37℃ with 5% CO_2. Strict aseptic techniques were followed when handling the cells, and all culture ware was sterilized prior to use.

Plasmids and siRNA

Plasmids containing HA-ubiquitin (wild-type: #17608; K48: #17605; K63: #17606), GFP-TRAF6 (#48711), FLAG-YAP1 (#66853) and YAP mutants were obtained from the Miao Ling plasmid platform (Miaoling Biological Technology, Wuhan, China). All constructs were confirmed by DNA sequencing before further application. The siRNA kit (*PPARα*, *TRAF6*, and *SKP2*) was obtained from the RiboBio (RiboBio, Guangzhou, China).

Transfection

For plasmid transfection, the cells were inoculated into 10 cm cell culture dishes at 70% density and then experimented with Lipofectamine 2000 (Thermo Fisher Scientific) according to the manufacturer's instructions.

For siRNA transfection, the cells were seeded into 6-well plates at 40% confluence. Cells were then transfected with gene-specific siRNA or control siRNA (20 nM) by using Lipofectamine RNAi MAX transfection reagent (Thermo Fisher Scientific) according to the manufacturer's instructions.

Immunoprecipitation and pulldown assays

Protein–protein interactions within the cells were analyzed based on previously reported methods [31]. Cells were washed with PBS before lysis in CHAPS buffer (120 mM NaCl, 20 mM NaF, 1 mM EDTA, 25 mM Tris–HCl, pH 7.5, 0.33% CHAPS) containing protease inhibitors (Roche). The lysates were centrifuged at 12,000 rpm for 10 min at 4 ℃. The supernatants were incubated with Flag antibody for 3 h at 4 ℃, followed by incubation with protein A/G agarose beads (Thermo Fisher Scientific) for another 6 h at 4 ℃. After washing three times with lysis buffer, the proteins were denatured in loading buffer containing 5% (v/v).

RNA isolation and qPCR

Cells were harvested in TRIzol (Invitrogen) for total RNA extraction, and cDNA was obtained by reverse transcription of RNA using TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix Kit (TransGen Biotech). SYBR Premix Ex TaqTMα (TaKaRa) was used to carry out real-time qPCR analyses with QuantStudio 6 Flex and QuantStudioTM Real-time PCR software. The primers used for human *YAP* mRNA transcription were as follows: 5′-CCT GCG TAG CCA GTT ACC AA-3′ (forward) and 5′-CCA TCT CAT CCA CAC TGT TC-3′ (reverse); for human *CTGF* mRNA transcription were as follows: 5′-CAG CAT GGA CGT TCG TCT G-3′ (forward) and 5′-AAC CAC GGT TTG GTC CTT GG-3′ (reverse); for human *CYR61*: 5′-CTC GCC TTA GTC GTC ACC C-3′ (forward) and 5′-CGC CGA AGT TGC ATT CCA G-3′ (reverse); for human *ACOX1*: 5′-GGA ACA ACG GAA AAG CGA GAA-3′ (forward) and 5′-GAA ACC TCG GCA CAT CCA CA-3′ (reverse); and for human *18 s*: 5′-GTG ACG TTG ACA TCC GTA AAG A-3′ (forward) and 5′-GCC GGA CTC ATC GTA CTC C-3′ (reverse). The fold changes in mRNA levels were calculated by the ΔΔCt method.

Analysis of protein ubiquitination

HepG2 cells were transfected with 5 μg YAP plasmid together with 5 μg HA-Ub, HA-Ub-K48, or HA-Ub-K63.

About 6 h later, cells were treated WY-14643 for 24 h. The cells were treated with 20 μM MG132 for 6 h before being harvested directly. The supernatant was collected and immunoprecipitated by FLAG antibody. Western blotting with HA antibody was used to detect the ubiquitinated of YAP.

Immunofluorescence staining

HepG2 cells were cultured on the cell climbing plate to the appropriate density, washed with PBS for 3 times, fixed with 4% paraformaldehyde for 15 min, and penetrated with 0.5% Triton X-100 at room temperature for 20 min. Normal goat serum was closed at room temperature for 30 min, and the slide was incubated with 1% BSA diluted primary antibody at 4℃ overnight. After washing with PBS, the slides were incubated with Alexa Fluor 488 coupled secondary antibody (1:100 dilution) for 1 h. DAPI was added to avoid light for 5 min, and then the slide was cleaned and installed. The acquired images are then viewed under a fluorescence microscope, and the images are processed using Image J.

Western blot

Standard western-blot assays were used to analyze protein expression in cells. The following antibodies were used for assays: anti-Flag (AE092, Abclonal, 1:1000), anti-HA (3724, CST, 1:1000), anti-YAP (14074, CST 1:1000), anti-CYR61 (14479, CST 1:1000), anti-CTGF (10095, CST, 1:1000), anti-ACOX1 (10957, Proteintech, 1:1000), and anti-β-Actin (AC038, Abclonal, 1:10,000). Protein signals were detected with an ECL kit (Millipore Corporation, USA) and immunoblotting intensity was quantified using Image J.

Statistical analysis

All statistical analyses were performed using GraphPad 8.0. The values were presented as means ± standard deviation (SD) and two-tailed unpaired Student's *t*-tests were used for statistical analysis. Results were considered significant when $P < 0.05$ ($^{*}P < 0.05$, $^{**}P < 0.01$).

Abbreviations

PPARα	Peroxisome proliferator-activated receptor α
PPREs	Peroxisome proliferator response elements
YAP	Yes-associated protein
HERC3	HECT domain and RCC1-like domain-containing protein 3
TRAF6	TNF receptor-associated factor 6
LATS1/2	Large tumor suppressor 1/2
MST1/2	Mammalian ste20-like kinases 1/2
CYR61	Cysteine-rich angiogenic inducer 61
CTGF	Connective tissue growth factor
ACOX1	Acyl-CoA oxidase 1
USP36	Ubiquitin-specific peptidase 36
SKP2	S-phase kinase-associated protein 2
PXR	Pregnane X receptor

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12915-025-02163-5.

Additional file 1. Figures S1-S4. Fig. S1—The expression of *PPARα* was detected by RT-qPCR. Fig. S2—Fenofibrate regulates YAP ubiquitination. Fig. S3—PPARα activation regulates K63-linked ubiquitination of YAP via TRAF6. Fig. S4—YAP interacts with PPARα through the WW domain.

Additional file 2. Unprocessed images of gels/blots for Figs. 1–4 and Figures S2-S4.

Acknowledgements

We thank our funders for the support.

Authors' contributions

H.B., S.F. and L.Y. conceived and designed the research. W.Z., S.Z., Y.C. and Z.C. performed the experiments and data analyses. W.Z. G.B., M.G. and X.J. collected the sample and interpreted the results. W.Z., S.F. and H.B. wrote the manuscript with input from all authors. W.Z., S.F., X.Y., J.F. and H.B. revised article. All authors contributed to discussions of the results and manuscript revision. All authors read and approved the final manuscript.

Funding

The work was supported by the Natural Science Foundation of China (Grant number: U23A20535, 82025034, 82304603), the National Key R&D Program of China (2022YFA1104900), the Guangdong Basic and Applied Basic Research Foundation (Grant number: 2023A1515012859), the Shenzhen Science and Technology Program (KQTD20190929174023858), the Science and Technology Innovation Project of Guangdong Medical Products Administration (Grant number: 2023ZDZ06), the Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (Grant number: 2017BT01Y093), the Guangdong Medical Research Foundation (Grant number: A2023109), the Foshan Medical Specialty Project (Grant number: FSZD145035) and the Open Project of Guangdong Provincial Key Laboratory of New Drug Screening.

Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors approved the final manuscript and the submission to this journal.

Competing interests

The authors declare no competing interests.

Received: 23 October 2024 Accepted: 17 February 2025
Published online: 28 February 2025

References

1. Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by eroxisome proliferators. Nature. 1990;347(6294):645–50.
2. Pawlak M, Lefebvre P, Staels B. Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. J Hepatol. 2015;62(3):720–33.
3. Tahri-Joutey M, Andreoletti P, Surapureddi S, Nasser B, Cherkaoui-Malki M, Latruffe N. Mechanisms mediating the regulation of peroxisomal fatty acid beta-oxidation by PPARα. Int J Mol Sci. 2021;22(16): 8969.
4. Berger J, Moller DE. The mechanisms of action of PPARs. Annu Rev Med. 2002;53:409–35.

5. Mirza AZ, Althagafi II, Shamshad H. Role of PPAR receptor in different diseases and their ligands: physiological importance and clinical implications. Eur J Med Chem. 2019;166:502–13.
6. Takada I, Makishima M. Peroxisome proliferator-activated receptor agonists and antagonists: a patent review (2014-present). Expert Opin Ther Pat. 2020;30(1):1–13.
7. Yang J, Yang X, Zhang YF, Tian JN, Fan SC, Gao Y, Li HL, Cai CH, Huang M, Bi HC. Peroxisome proliferator-activated receptor α agonist induces mouse hepatomegaly through the spatial hepatocyte enlargement and proliferation. Acta Pharmacol Sin. 2023;44(10):2037–47.
8. Feige JN, Gelman L, Michalik L, Desvergne B, Wahli W. From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions. Prog Lipid Res. 2006;45(2):120–59.
9. Yu FX, Zhao B, Guan KL. Hippo pathway in organ size control, tissue homeostasis, and cancer. Cell. 2015;163(4):811–28.
10. Kodaka M, Hata Y. The mammalian Hippo pathway: regulation and function of YAP1 and TAZ. Cell Mol Life Sci. 2015;72(2):285–306.
11. Kofler M, Kapus A. Nuclear import and export of YAP and TAZ. Cancers (Basel). 2023;15(20):4956.
12. Pobbati AV, Hong W. A combat with the YAP/TAZ-TEAD oncoproteins for cancer therapy. Theranostics. 2020;10(8):3622–35.
13. Fan S, Gao Y, Qu A, Jiang Y, Li H, Xie G, Yao X, Yang X, Zhu S, Yagai T, Tian J, Wang R, Gonzalez FJ, Huang M, Bi H. YAP-TEAD mediates PPARα-induced hepatomegaly and liver regeneration in mice. Hepatology. 2022;75(1):74–8.
14. Fan S, Gao Y, Zhao P, Xie G, Zhou Y, Yang X, Li X, Zhang S, Gonzalez FJ, Qu A, Huang M, Bi H. Fenofibrate-promoted hepatomegaly and liver regeneration are PPARα-dependent and partially related to the YAP pathway. Acta Pharm Sin B. 2024;14(7):2992–3008.
15. Swatek KN, Komander D. Ubiquitin modifications. Cell Res. 2016;26(4):399–422.
16. Sun T, Liu Z, Yang Q. The role of ubiquitination and deubiquitination in cancer metabolism. Mol Cancer. 2020;19(1):146.
17. Yuan B, Liu J, Shi A, Cao J, Yu Y, Zhu Y, Zhang C, Qiu Y, Luo H, Shi J, Cao X, Xu P, Shen L, Liang T, Zhao B, Feng XH. HERC3 promotes YAP/TAZ stability and tumorigenesis independently of its ubiquitin ligase activity. EMBO J. 2023;42(4): e111549.
18. Liu M, Yan M, Lv H, Wang B, Lv X, Zhang H, Xiang S, Du J, Liu T, Tian Y, Zhang X, Zhou F, Cheng T, Zhu Y, Jiang H, Cao Y, Ai D. Macrophage K63-linked ubiquitination of YAP promotes its nuclear localization and exacerbates atherosclerosis. Cell Rep. 2020;32(5): 107990.
19. Zhang W, Luo J, Xiao Z, Zang Y, Li X, Zhou Y, Zhou J, Tian Z, Zhu J, Zhao X. USP36 facilitates esophageal squamous carcinoma progression via stabilizing YAP. Cell Death Dis. 2022;13(12):1021.
20. Driskill JH, Pan D. The Hippo Pathway in liver homeostasis and pathophysiology. Annu Rev Pathol. 2021;16:299–322.
21. Eldeeb MA, Siva-Piragasam R, Ragheb MA, Esmaili M, Salla M, Fahlman RP. A molecular toolbox for studying protein degradation in mammalian cells. J Neurochem. 2019;151(4):520–33.
22. Yao F, Zhou Z, Kim J, Hang Q, Xiao Z, Ton BN, Chang L, Liu N, Zeng L, Wang W, Wang Y, Zhang P, Hu X, Su X, Liang H, Sun Y, Ma L. SKP2- and OTUD1-regulated non-proteolytic ubiquitination of YAP promotes YAP nuclear localization and activity. Nat Commun. 2018;9(1):2269.
23. Li JA, Kuang T, Pu N, Fang Y, Han X, Zhang L, Xu X, Wu W, Wang D, Lou W, Rong Y. TRAF6 regulates YAP signaling by promoting the ubiquitination and degradation of MST1 in pancreatic cancer. Clin Exp Med. 2019;19(2):211–8.
24. Yan F, Qian M, He Q, Zhu H, Yang B. The posttranslational modifications of Hippo-YAP pathway in cancer. Biochim Biophys Acta Gen Subj. 2020;1864(1): 129397.
25. Zhao B, Li L, Tumaneng K, Wang CY, Guan KL. A coordinated phosphorylation by Lats and CK1 regulates YAP stability through SCF(beta-TRCP). Genes Dev. 2010;24(1):72–85.
26. Oka T, Mazack V, Sudol M. Mst2 and Lats kinases regulate apoptotic function of Yes kinase-associated protein (YAP). J Biol Chem. 2008;283(41):27534–46.
27. Deng L, Meng T, Chen L, Wei W, Wang P. The role of ubiquitination in tumorigenesis and targeted drug discovery. Signal Transduct Target Ther. 2020;5(1):11.
28. Mo H, Li R, Yang N, Han J, Xiao X, Zhang Y, Xiao Z, Jiao L, Xu Q, Tu K. USP40 promotes hepatocellular carcinoma progression through a YAP/USP40 positive feedback loop. Cancer Lett. 2024;589: 216832.
29. Wang L, Liu X, Han Y, Tsai HI, Dan Z, Yang P, Xu Z, Shu F, He C, Eriksson JE, Zhu H, Chen H, Cheng F. TRAF6 enhances PD-L1 expression through YAP1-TFCP2 signaling in melanoma. Cancer Lett. 2024;590: 216861.
30. Bertini E, Oka T, Sudol M, Strano S, Blandino G. YAP: at the crossroad between transformation and tumor suppression. Cell Cycle. 2009;8(1):49–57.
31. Zhang S, Guo M, Jiang X, Tang L, Wu T, Bi G, Yang X, Fan S, Bi H. PXR triggers YAP-TEAD binding and Sirt2-driven YAP deacetylation and polyubiquitination to promote liver enlargement and regeneration in mice. Pharmacol Res. 2023;188: 106666.

Publisher's Note