Histol Histopathol (2024) 39: 1079-1087

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORIGINAL ARTICLE Open Access

Transcription factor YY1 accelerates hepatic fibrosis development by activating NLRP3 inflammasome-mediated pyroptosis

Xiao Fu[1], Ping Xiao[1], Xin Luo[1] and Ninghong Guo[2]

[1]General Medicine Department and [2]Clinical Trial Center, the Second Affiliated Hospital of Nanchang University, Nanchang, Jiangxi Province, PR China

Summary. Hepatic fibrosis is the basis of multiple liver diseases and may eventually develop into hepatocellular carcinoma. Hepatic stellate cell (HSC) activation is a driving factor of hepatic fibrogenesis. In the liver microenvironment, liver cells and others play a crucial role in HSC activation. The liver tissues of CCl4-induced rats show excessive fibrosis, inflammation, and cell apoptosis. Yin Yang 1 (YY1) was highly expressed in hepatic fibrosis rats and TGF-β1-treated liver cells. In animal experiments, YY1 knockdown effectively attenuated CCl_4-induced liver injury and pyroptosis-related IL-1β and IL-18 expression. In cellular experiments, NLRP3 inflammasome-mediated pyroptosis was activated by TGF-β1 treatment, while YY1 knockdown significantly inhibited the activation of the NLRP3 inflammasome, pyroptosis, and the secretion of IL-1β and IL-18. In addition, our data showed that TGF-β1-treated liver cell conditional medium markedly induced HSC activation, which was rescued by YY1 knockdown in liver cells. YY1 overexpression in liver cells contributed to the activation of TGF-β1-treated liver cell conditional medium in HSCs, however, this effect of YY1 was attenuated by NLRP3 inhibition. Overall, YY1 overexpression in liver cells contributed to HSC activation by facilitating IL-1β and IL-18 production via activating NLRP3 inflammasome-mediated pyroptosis, thus aggravating hepatic fibrogenesis. Our data indicate that YY1 may be a novel target for the treatment of hepatic fibrosis and associated liver diseases.

Key words: Hepatic fibrosis, Pyroptosis, Yin Yang 1, Hepatic stellate cells

Introduction

Hepatic fibrosis is the last stage of many chronic liver disorders, which accounts for two million deaths globally each year, being a considerable economic burden on society. Hepatic fibrosis can develop into cirrhosis, eventually causing end-stage hepatocellular carcinoma, and can be induced by numerous factors like hepatitis B/C virus infection, hemochromatosis, non-alcoholic steatohepatitis, autoimmune hepatitis, non-alcoholic fatty liver disease, and alcohol abuse (Xu et al., 2016; Higashi et al., 2017; Gheorghe et al., 2021). Hepatic stellate cell (HSC) activation is a key event in hepatic fibrogenesis. Activated HSCs differentiate into myofibroblasts, resulting in extracellular matrix (ECM) deposition and fibrogenesis (Dewidar et al., 2019; Roehlen et al., 2020). It was demonstrated that the liver microenvironment affects the activation of HSCs. Many cells in the liver microenvironment can crosstalk with HSCs, such as liver cells, macrophages, T cells, and sinusoidal endothelial cells (Yang et al., 2021). Clarification of the cellular communication of HSCs and other cells will provide valuable ideas for the treatment of hepatic fibrosis.

Inflammation is an important enabler of hepatic fibrogenesis. Pyroptosis is a pro-inflammatory cell death, which is activated by the inflammasome and Gasdermin D N-terminal fragment (GSDMD-N) during the development of hepatic fibrosis (Zhang et al., 2020). The NOD-like receptor family pyrin domain containing 3 (NLRP3), ASC, and pro-caspase-1 constitute the NLRP3 inflammasome. It was reported that activated NLRP3 inflammasomes could induce pyroptosis and thus increase the production of interleukin (IL)-1β and

Corresponding Author: Ninghong Guo, Clinical Trial Center, the Second Affiliated Hospital of Nanchang University, No. 1, Minde Road, Donghu District, Nanchang 330006, Jiangxi Province, PR China. e-mail: guoninghong1122@163.com

www.hh.um.es. DOI: 10.14670/HH-18-703

Abbreviations. α-SMA, alpha smooth muscle actin; CCl_4, Carbon tetrachloride; ECM, extracellular matrix; GSDMD-N, Gasdermin D N-terminal fragment; HSCs, hepatic stellate cells; IL, interleukin; NLRP3, NOD-like receptor family pyrin domain containing 3; TGF-β1, transforming growth factor beta 1; YY1, Yin Yang 1.

IL-18 (Koh et al., 2021; Yu et al., 2021). During the development of hepatic fibrosis, NLRP3 inflammasome activation leads to liver cell pyroptosis, which was proven to contribute to liver inflammation and hepatic fibrogenesis (Wree et al., 2014). It was indicated that liver cells undergoing pyroptosis could induce HSC activation and hepatic fibrosis by releasing NLRP3 inflammasome particles and pro-inflammatory cytokines IL-1β (Gaul et al., 2021). Previous studies suggested that inhibiting liver cell pyroptosis may be a practicable method to suppress the activation of HSCs.

To explore the mechanism by which the *NLRP3* inflammasome is activated in hepatic fibrosis, we predicted the transcription factors that bind to the promoter of the *NLRP3* gene by using the JASPAR (https://jaspar.elixir.no/) and PROMO databases (https://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3). We found that Yin Yang 1 (YY1) can bind to multiple sites on the promoter of NLRP3. The transcription factor YY1 belongs to the zinc finger protein family and can facilitate or suppress target gene expression by recognizing a specific consensus sequence. It was demonstrated that YY1 is involved in a series of biological processes like proliferation, inflammation, apoptosis, and fibrogenesis (Gordon et al., 2006; Ou et al., 2019). Zhang et al. reported that upregulation of YY1 in alveolar epithelial cells aggravates transforming growth factor beta 1 (TGF-β1)-induced epithelial-mesenchymal transition and is associated with the pro-fibrogenesis phenotype of the cells (Zhang et al., 2019). Recently, Liu et al. revealed that inhibition of YY1 in HSCs could effectively inhibit HSC proliferation and repress fibrosis-related factors, alpha-smooth muscle actin (α-SMA), and collagen deposition, indicating the promotor effect of YY1 in hepatic fibrosis (Liu et al., 2019). However, the regulatory mechanism of YY1 in hepatic fibrogenesis remains unclear. Here, we probed whether YY1 is involved in liver cell pyroptosis and HSC activation during hepatic fibrogenesis and its action mechanism.

Materials and methods

Animal experiments

Eight-week-old SPF-grade Wistar rats (male), obtained from the SYSU animal center (Guangzhou, China), weighing 200~250 g, were used. Animal housing conditions included water and food *ad libitum*, a suitable temperature of 25°C, and 12-hour cycles of light/dark. All rats were raised in an SPF-grade experimental animal feeding center for seven days and were then utilized for study. Animal protocols were approved by the Animal Care and Use Committee of the Second Affiliated Hospital of Nanchang University. Animal experiments were performed according to the confirmed guidelines.

A hepatic fibrosis rat model was generated by carbon tetrachloride (CCl_4) administration. Rats were randomly divided into two or four groups (n=6 per group). Two groups: Control group and CCl_4 group; Four groups: Control group, Model group (CCl_4 group), Model + Lv-sh-NC group, and Model + Lv-sh-YY1 group.

Model rats were intraperitoneally administered 30% CCl_4 (1.5 ml/kg, mixed with olive oil; Sinopharm, Beijing, China) twice weekly. The rats in the Control group were intraperitoneally administered an equal volume of olive oil. The day following the first CCl_4 administration, some model rats were injected in the tail vein with lentivirus (Genechem Company, Shanghai, China) expressing YY1 shRNA (sh-YY1) or negative control shRNA (sh-NC). Eight weeks after CCl_4 administration, all rats were anesthetized using 60 mg/kg pentobarbital (Sigma-Aldrich, USA), and then liver tissues were obtained from each rat for further analysis, including RT-qPCR, Western blotting, ELISA assay, Sirius Red staining, HE staining, and TUNEL staining.

Sirius Red, HE, and TUNEL staining

Liver tissues were cleaned using normal saline and fixed with 10% formalin for 48h and were then embedded in paraffin and prepared into 4-μm sections. These sections were deparaffinized in xylene and rehydrated in diluted alcohol. Afterward, fibrosis, inflammation, and cell apoptosis in liver tissues were analyzed using Sirius Red, HE, and TUNEL staining, respectively. Finally, five random microscope fields for each liver section were obtained for analysis with Image J software. The Sirius Red, HE, and TUNEL staining kits were obtained from Solarbio Company (Beijing, China). All experiments were carried out in accordance with the manufacturer's protocols.

Cell culture and treatment

Four types of cells were utilized in the current study, including primary HSCs isolated from normal rats, primary HSCs isolated from model rats, human liver cell line QSG-7701 (American Type Culture Collection, MD, USA), and human immortalized HSC line LX-2 (Be Na Culture Collection, Beijing, China). Primary HSCs were isolated from the liver tissues of rats according to a previous study (Jiang et al., 2017) and were grown in Rat HSC cell culture medium (Pricella, Wuhan, China). QSG-7701 and LX-2 cells were grown in complete Dulbecco's modified Eagle's medium (DMEM), composed of DMEM (Gibco, NY, USA), 10% fetal bovine serum (FBS; HyClone, GE, USA), streptomycin (100 μg/ml; Gibco) and penicillin (100 U/ml; Gibco). Cell culture was performed in a humidified 5% CO_2 incubator at 37°C.

In the current study, QSG-7701 was treated with 10 ng/ml of TGF-β1 (Sigma-Aldrich) to mimic hepatic fibrosis-induced cell injury. pcDNA 3.1 vector, pcDNA 3.1 vector expressing YY1, YY1 siRNA (si-YY1; GenePharma Company), and negative control siRNA (si-

NC; GenePharma Company) were transfected into QSG-7701 cells utilizing Lipofectamine™2000 reagent (Invitrogen, NY, USA). In addition, QSG-7701 cells were treated with 1 μM of MCC950 to suppress NLRP3 and NLRP3 inflammasome-mediated pyroptosis. At 48h after transfection or MCC950 treatment, cells were collected for qRT-PCR or Western blotting, and cell supernatants were collected for ELISA assay.

Moreover, in order to determine the influence of liver cells on HSC activation and the role of YY1 in this process, we cultured LX-2 cells using the mixture of specific QSG-7701 cell conditional medium and fresh complete DMEM. QSG-7701 cells were treated with TGF-β1 alone or in combination with si-YY1, YY1-overexpression vector, or MCC950. At 48h after transfection or treatment, cell culture supernatants from each group of QSG-7701 cells were collected and then utilized for LX-2 cell culture. After 24h conditional culture, LX-2 cells were collected for the next detection.

Detection of gene expression

qRT-PCR was carried out to detect mRNA expression of YY1, α-SMA, IL-1β, IL-18, and Collagen III in liver cells, HSCs, and liver tissues. Total RNA was extracted from cells and liver tissues utilizing TRIzol reagent (Invitrogen) and was then reverse transcribed into complementary DNA through a Transcriptor First Strand cDNA Synthesis Kit (TaKaRa, Shiga, Japan). Subsequently, qRT-PCR was performed to measure the relative expression of each mRNA utilizing the SYBR Green mix (TaKaRa) on a real-time PCR machine (MX3000p, Agilent). *GAPDH* served as an internal reference for the qRT-PCR assay. The relative expression of these mRNAs was ensured through the $2^{-\Delta\Delta Ct}$ method.

Detection of protein expression

Western blot was carried out to analyze protein expression. Total protein was isolated from cells or tissues utilizing RIPA lysis buffer (Invitrogen); subsequently, the protein concentration of each sample was measured with a ThermoFisher NanoDrop 2000 spectrophotometer. After that, 25 μg of equal-quality protein samples from each group were loaded and separated on a 12% SDS-PAGE and then transferred onto a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% non-fat milk for 1h at room temperature and incubated with diluted primary antibodies overnight at 4°C. The primary antibodies (Abcam, USA) included anti-YY1, anti-α-SMA, anti-Collagen III, anti-NLRP3, anti-caspase-1 p20, anti-GSDMD-N, and anti-GAPDH, which were diluted 1:2000 with 5% non-fat milk. GAPDH served as an internal reference. Next, the membrane was incubated with goat anti-mouse or anti-rabbit secondary antibodies (Abcam) for 1h at room temperature. Finally, a chemiluminescence reagent (Solarbio) was utilized to visualize the protein bands, and the relative expression of protein was analyzed by utilizing Image J software.

Detection of cytokine production

ELISA assay was performed to measure levels of IL-1β and IL-18 in liver tissues or the supernatants of QSG-7701 cells. This experiment was accomplished by utilizing a rat/human IL-1β ELISA kit (Sigma-Aldrich) and a rat/human IL-18 ELISA kit (Solarbio). These experiments were performed in accordance with the kits' protocols.

Cell viability assay

LX-2 cells were seeded into 96-well plates at a density of $5x10^3$ cells/well and were cultured in the conditional medium of QSG-7701 cells. At 24h after cell culture, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) solution (Promega, WI, USA) was added into each well (20 μl per well) for another 2h. Then, formazan crystals were solubilized with DMSO solution. The absorbance at 490 nm was measured with an ELISA reader (BMG, Offenburg, Germany).

Statistical analyses

Data analyses were carried out using Graph Prism 7.0 (GraphPad Software); all continuous data were presented as mean ± standard deviation. The significant difference between two independent groups was ensured by the Student's t-test, and the significant difference among groups (≥3 groups) was ensured by one-way analyses of variance followed by post hoc comparison. All data were obtained from five biological replicates. $P<0.05$ was considered statistically significant.

Results

YY1 was increased in hepatic fibrosis

To detect YY1 expression in hepatic fibrosis, a hepatic fibrosis rat model was generated via CCl4 administration. Our data showed that the expression of α-SMA-a marker of fibrosis-at both mRNA and protein levels, was higher in liver tissues of hepatic fibrosis rats than in those of normal rats (Fig. 1A,B). The YY1 mRNA level was higher in the liver tissues of hepatic fibrosis rats than in those of normal rats (Fig. 1A). The YY1 protein level in liver tissues of hepatic fibrosis rats was also higher than in liver tissues of normal rats (Fig. 1B). Subsequently, we isolated primary HSCs from healthy rats and hepatic fibrosis rats. As shown in Figure 1C, α-SMA and YY1 expression were markedly increased in primary HSCs isolated from hepatic fibrosis rats compared with those isolated from normal rats. In primary HSCs isolated from normal rats, the expression of α-SMA and YY1 was upregulated with TGF-β1 induction (Fig. 1D). In summary, YY1 was increased in

hepatic fibrosis.

Silencing of YY1 could significantly attenuate hepatic fibrosis

In order to clarify whether YY1 could be a candidate target for the treatment of hepatic fibrosis, we interfered with YY1 expression in hepatic fibrosis rats by utilizing a lentivirus expressing YY1 shRNA (Lv-sh-YY1). Excessive fibrosis in liver tissues of hepatic fibrosis rats was examined by Sirius Red staining, which was partly relieved by YY1 silencing (Fig. 2A,B). Compared with normal rats, the liver tissues of hepatic fibrosis rats showed inflammatory infiltration and tissue damage. Following YY1 silencing, the inflammatory infiltration and liver tissue damage in hepatic fibrosis rats were attenuated (Fig. 2C,D). Also, the TUNEL-positive cell number was increased in the liver tissues of hepatic fibrosis rats. YY1 silencing could markedly reduce TUNEL-positive cells in hepatic fibrosis rats (Fig. 2E,F). The expression of α-SMA mRNA (Fig. 2G) and protein (Fig. 2H) in the liver tissues of hepatic fibrosis rats was also increased, which was partly rescued following YY1 silencing. We then detected the production of inflammatory cytokines (IL-1β and IL-18) in hepatic fibrosis rats. As the results of qRT-PCR and ELISA assay, expression of IL-1β mRNA (Fig. 2I) and IL-18 mRNA (Fig. 2J) and the production of IL-1β (Fig. 2L) and IL-18 (Fig. 2K) were significantly upregulated in the liver tissues of hepatic fibrosis rats when contrasted against normal rats, while the upregulation of IL-1β and IL-18 in hepatic fibrosis rats was effectively downregulated by YY1 silencing. Overall, YY1 silencing could effectively attenuate fibrosis, inflammation, and liver damage in hepatic fibrosis rats.

YY1 silencing suppressed NLRP3 inflammasome-mediated pyroptosis

Pyroptosis is an inflammation-related method of cell death that plays a crucial role in the development of hepatic fibrosis. To investigate the regulation of YY1 in NLRP3 inflammasome-mediated pyroptosis in hepatic fibrosis, we silenced the expression of YY1 in the TGF-β1-induced human liver cell line QSG-7701. Our data illustrated that the expression of YY1 was increased in TGF-β1-induced QSG-7701, however, the TGF-β1-induced increase in YY1 was effectively suppressed by YY1 siRNA transfection (Fig. 3A). Importantly, TGF-β1 treatment evidently upregulated IL-1β mRNA (Fig. 3B) and IL-18 mRNA (Fig. 3C) expression in QSG-7701 cells, which was rescued by YY1 siRNA transfection. NLRP3 expression and the expression of pyroptosis-related factors like caspase-1 and GSDMD-N were increased in TGF-β1-induced QSG-7701 cells, while YY1 silencing markedly downregulated the expression of these proteins in TGF-β1-induced QSG-7701 cells (Fig. 3D). Furthermore, the TGF-β1-induced increase in IL-1β (Fig. 3E) and IL-18 (Fig. 3F) production in the supernatants of QSG-7701 cells was partly rescued by YY1 siRNA transfection. In conclusion, NLRP3 inflammasome-mediated pyroptosis was inactivated by YY1 silencing.

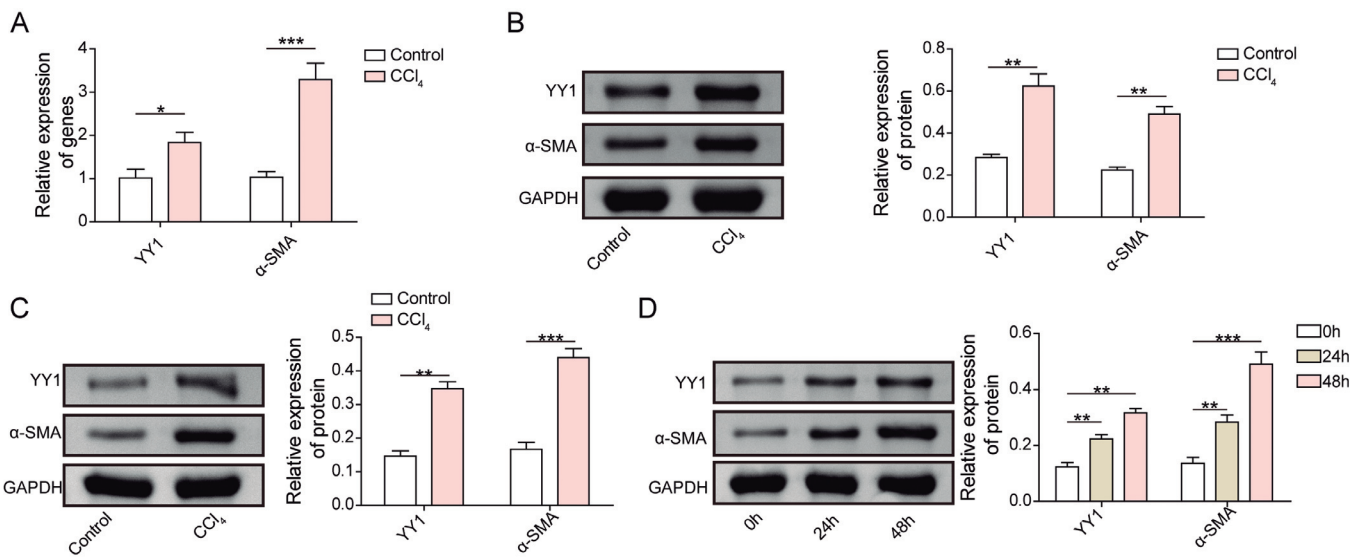

Fig. 1. Expression of YY1 and α-SMA in hepatic fibrosis rats and TGF-β1-induced liver cells. Rats were randomly divided into two groups: Control group and CCl_4 (Model) group. At the end of the modeling period, liver tissues were collected from each rat. **A.** Expression of YY1 and α-SMA mRNA in tissues was measured by RT-qPCR. **B.** Expression of YY1 and α-SMA protein in tissues was measured by Western blot. Subsequently, primary HSCs were isolated from the liver tissues of normal and hepatic fibrosis rats. **C.** Expression of YY1 and α-SMA protein in the cells was measured by Western blot. After that, primary HSCs isolated from normal rats were treated with 10 ng/ml TGF-β1 for 24 or 48h. **D.** Expression of YY1 and α-SMA protein in the cells was measured by Western blot. **P*<0.05, ***P*<0.01, ****P*<0.001.

YY1 silencing in QSG-7701 cells could suppress HSC activation

Activation of HSCs is a key event in hepatic fibrosis. Cellular crosstalk between HSCs and liver cells or other surrounding cells is helpful for the activation of HSCs (Yang et al., 2021). Our data indicated that TGF-β1-treated QSG-7701 cell conditional medium markedly

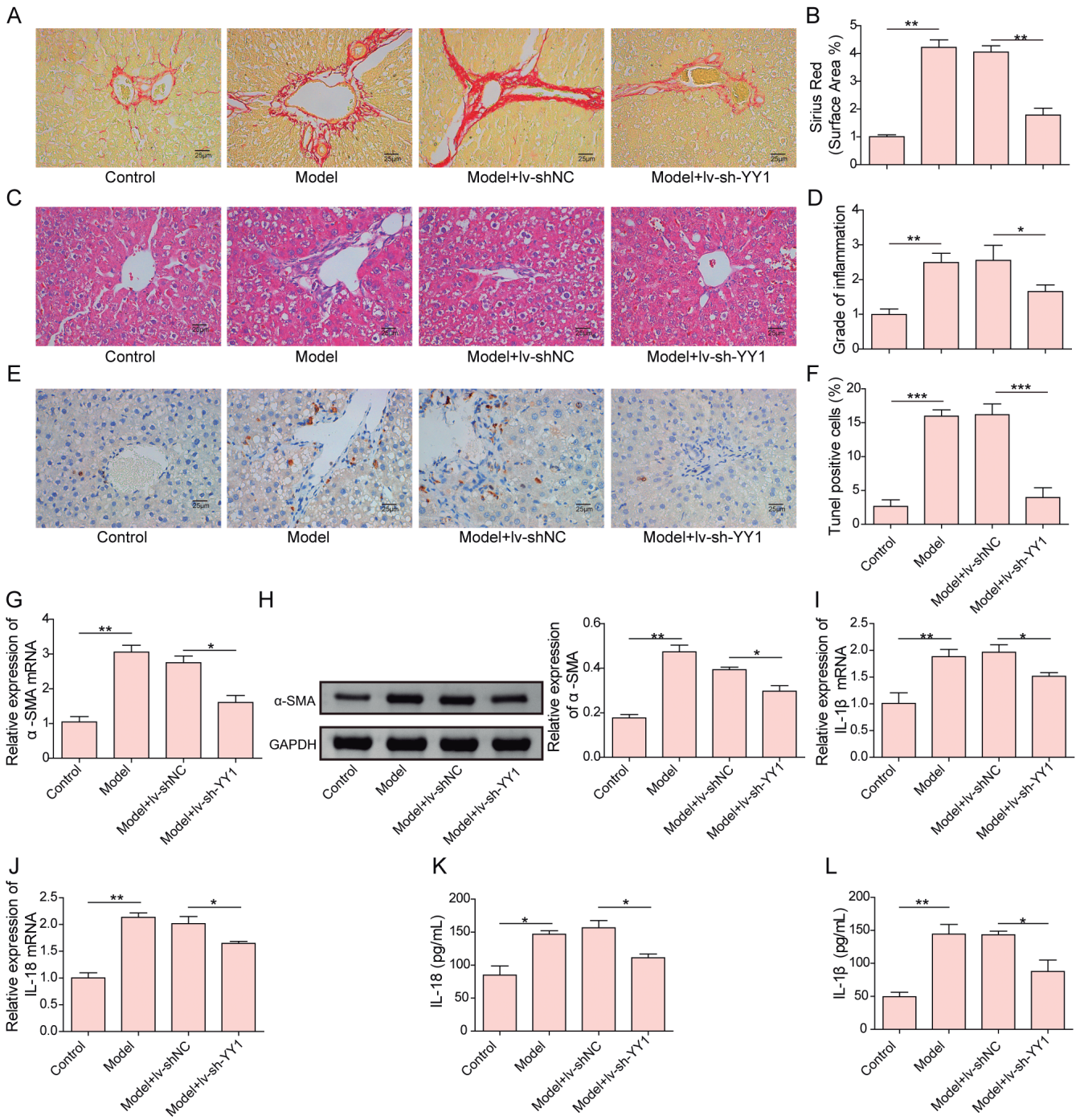

Fig. 2. Influences of YY1 knockdown on liver injury in hepatic fibrosis rats. Some hepatic fibrosis rats were injected with the lentivirus expressing YY1 shRNA or negative control shRNA. At the end of the CCl_4 treatment period, liver tissues were collected from each rat. **A, B.** Sirius Red staining was performed to determine fibrotic levels in the liver tissues. **C, D.** HE staining was carried out to examine inflammation and tissue injury in the liver tissues. **E, F.** TUNEL staining was performed to determine cell apoptosis in the liver tissues. **G.** Expression of α-SMA mRNA in the tissues was measured by RT-qPCR. **H.** Expression of α-SMA protein in the tissues was measured by Western blot. **I, J.** Expression of IL-1β and IL-18 mRNA in the tissues was measured by RT-qPCR. **K, L.** Production of IL-1β and IL-18 in the tissues was measured by ELISA assay. **P*<0.05, ***P*<0.01, ****P*<0.001. Scale bars: 25 μm.

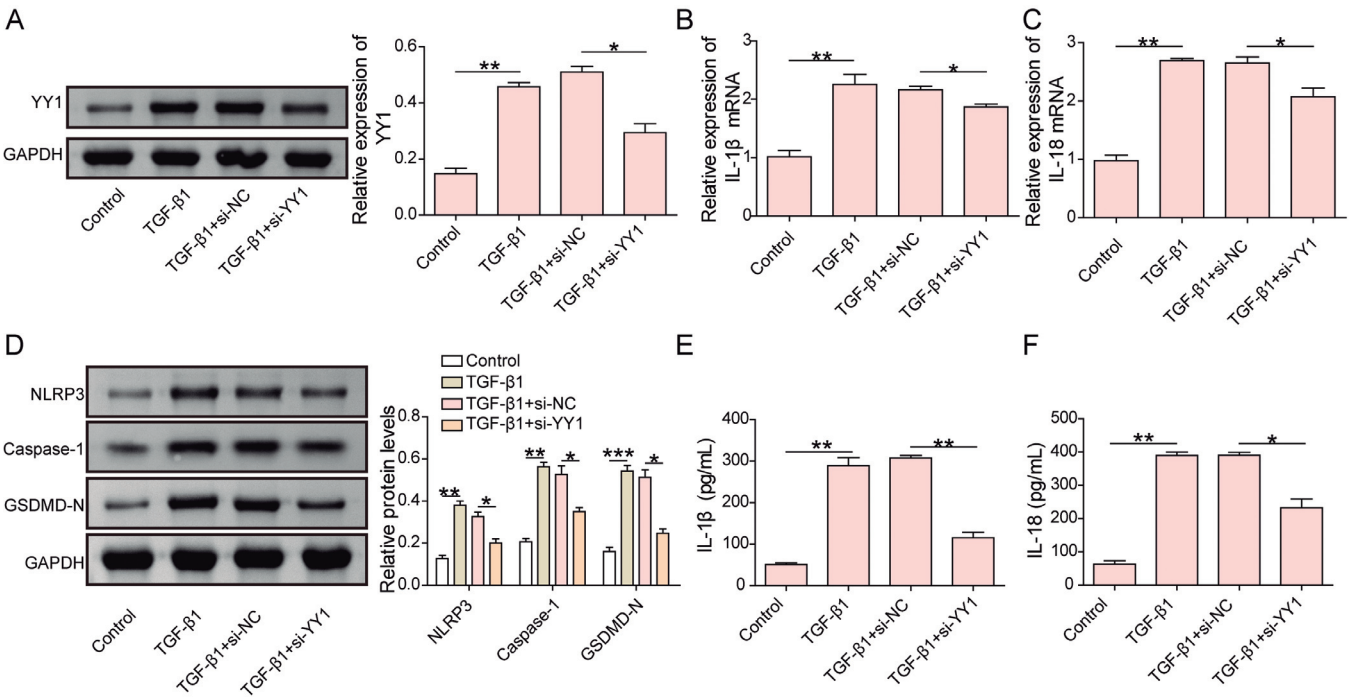

Fig. 3. Influences of YY1 knockdown on TGF-β1-induced pyroptosis in liver cells. Human liver cells QSG-7701 were treated with 10 ng/ml TGF-β1 or PBS for 48h. A part of the TGF-β1-treated cells were transfected with YY1 siRNA or negative control siRNA at the same time. **A.** Western blot was carried out to determine the expression of YY1 in the cells. **B, C.** Expression of IL-1β and IL-18 mRNA in the cells was analyzed by RT-qPCR. **D.** Western blot was carried out to determine the expression of NLRP3 inflammasome- and pyroptosis-associated factors, including NLRP3, caspase-1, and GSDMD-N. **E, F.** Secretion of IL-1β and IL-18 in the medium supernatant was measured by ELISA assay. **P*<0.05, ***P*<0.01, ****P*<0.001.

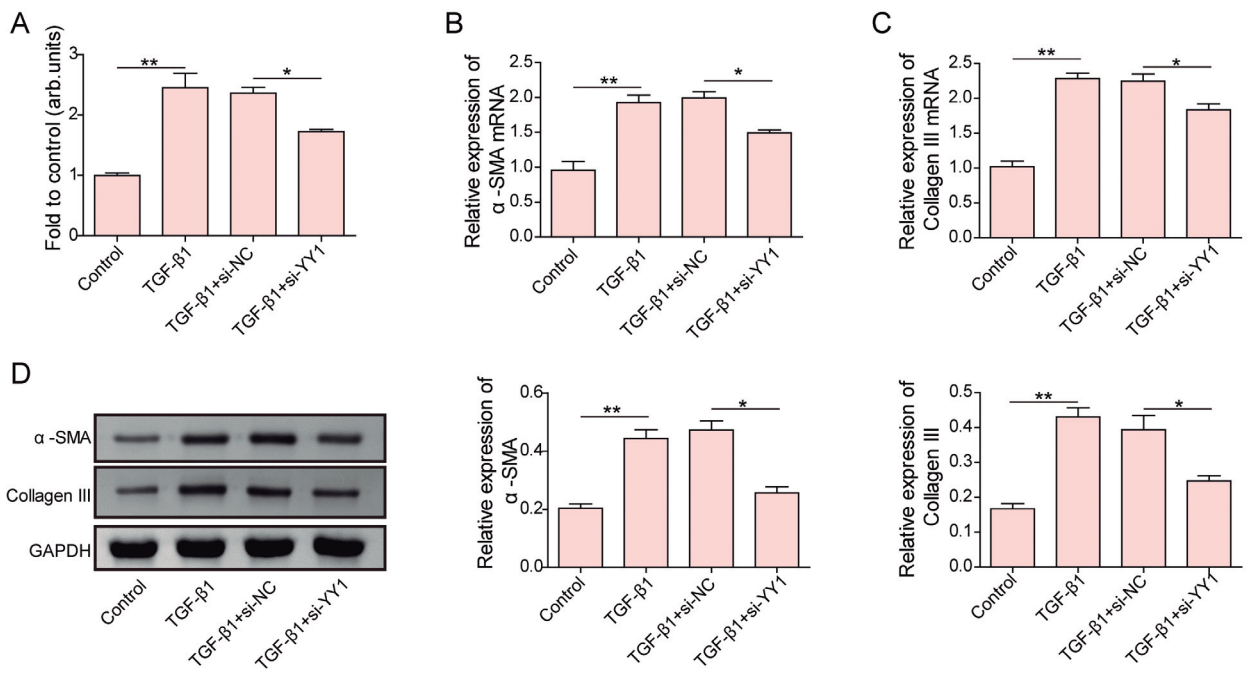

Fig. 4. YY1 knockdown suppressed HSC activation. QSG-7701 cells were treated with 10 ng/ml TGF-β1 or PBS. At the same time, TGF-β1-treated cells were transfected with YY1 siRNA or negative control siRNA. At 48h after TGF-β1 treatment and cell transfection, the cell culture medium of these cells was collected and mixed with fresh DMEM medium. This medium mixture was utilized to culture LX-2 for 24h. **A.** Cell viability of LX-2 cells was measured by MTT assay. **B, C.** Expression of α-SMA and Collagen III (a component of the ECM) mRNA in LX-2 cells was analyzed by RT-qPCR. **D.** Expression of α-SMA and Collagen III protein in LX-2 cells was analyzed by Western blot. **P*<0.05, ***P*<0.01, ****P*<0.001.

boosted HSC viability, however, the increase in HSC viability was constrained following YY1 siRNA transfection (Fig. 4A). Moreover, TGF-β1-treated QSG-7701 cell conditional medium significantly facilitated α-SMA and Collagen III expression at both the mRNA and protein levels. Compared with HSCs cultured with TGF-β1-treated QSG-7701 cell conditional medium, the expression of α-SMA and Collagen III mRNA and protein was downregulated in HSCs cultured with YY1 silenced-QSG-7701 cell conditional medium (Fig. 4B-D).

YY1 activated HSCs by inducing pyroptosis in liver cells

Based on the above results, we aimed to elucidate the relationship between YY1 and NLRP3; using the JASPAR database, we found binding sites between YY1 and *NLRP3* (Fig. 5A). Meanwhile, we clarified

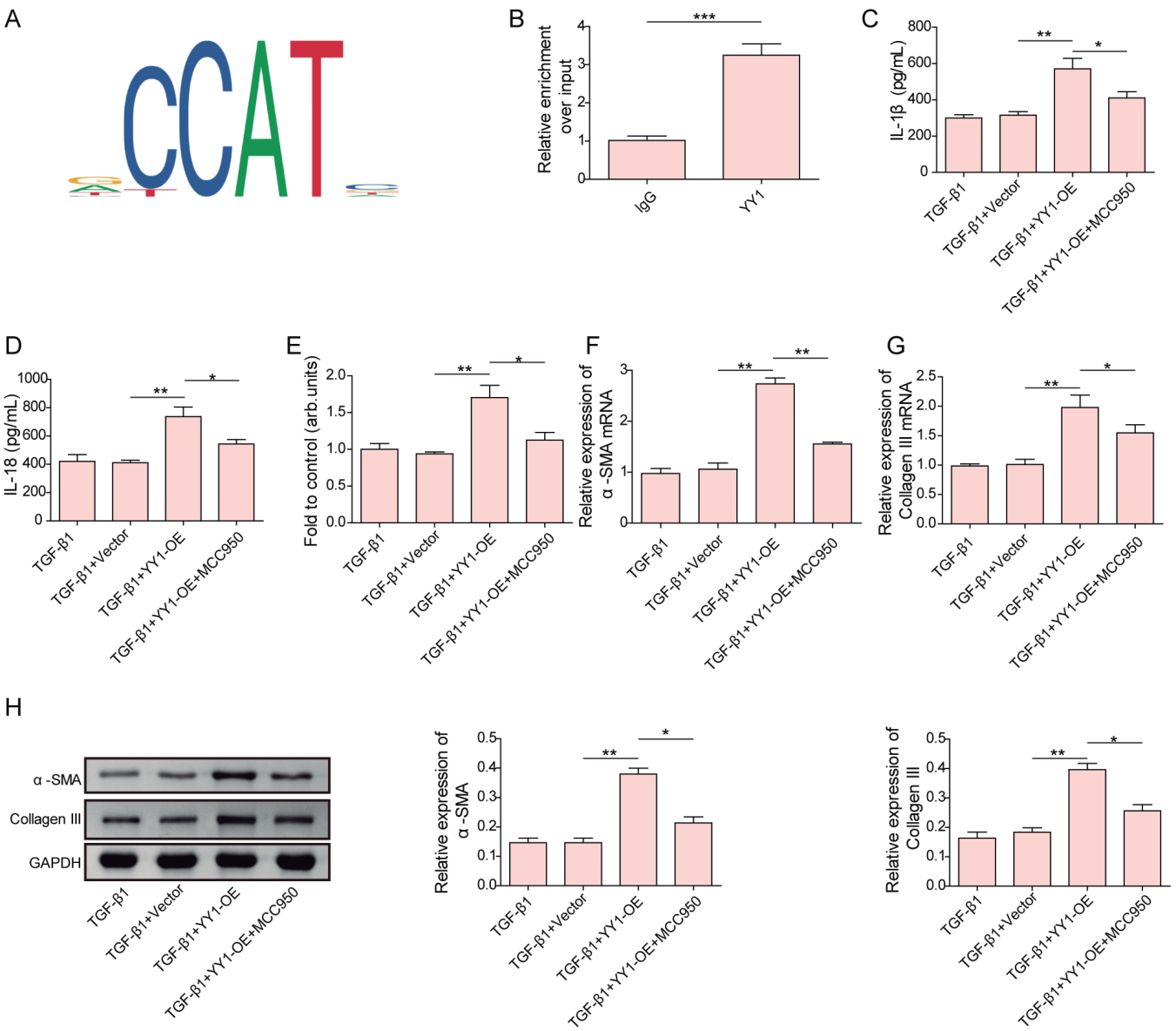

Fig. 5. Effects of NLRP3 inhibition on the promotion of YY1 to HSC activation. **A.** Binding sites between YY1 and NLRP3 in the JASPAR database. **B.** Combination of YY1 and NLRP3 was measured by Co-IP assay. QSG-7701 cells were treated with 10 ng/ml TGF-β1. At the same time, TGF-β1-treated cells were transfected with a YY1 overexpression vector or empty vector. Some YY1-overexpressed TGF-β1-treated QSG-7701 cells were administered NLRP3 inflammasome inhibitor MCC950. At 48h after drug treatment and cell transfection, IL-1β levels in the supernatant were measured by ELISA assay **(C)**, and IL-18 levels in the supernatant were also measured by ELISA assay **(D)**. Subsequently, the culture medium of these cells was collected and mixed with fresh DMEM medium. Medium mixtures were utilized to culture LX-2 for 24h. **E.** Cell viability of LX-2 cells was measured by MTT assay. **F, G.** Expression of α-SMA and Collagen III mRNA in LX-2 cells was analyzed by RT-qPCR. **H.** Expression of α-SMA and Collagen III protein in LX-2 cells was analyzed by Western blot. **P*<0.05, ***P*<0.01, ****P*<0.001.

the combination of YY1 and *NLRP3* (Fig. 5B). To probe whether YY1 induces HSC activation by regulating NLRP3-mediated pyroptosis, we treated TGF-β1-induced QSG-7701 cells with the YY1 overexpression vector and/or MCC950 (an NLRP3 inflammasome inhibitor). As shown in Figure 5C,D, in TGF-β1-treated QSG-7701, increasing YY1 could facilitate IL-1β and IL-18 production, which was rescued by treatment with MCC950. In addition, our results uncovered that YY1-overexpressed QSG-7701 cell conditional medium significantly accelerated HSC proliferation, which was rescued by pyroptosis inhibition (Fig. 5E). Compared with TGF-β1-treated QSG-7701 cell conditional medium, YY1-overexpressed QSG-7701 cell conditional medium facilitated α-SMA mRNA (Fig. 5F), Collagen III mRNA (Fig. 5G), and α-SMA and Collagen III protein expression (Fig. 5H) in HSCs, while their expression was partly attenuated by pyroptosis inhibition. Overall, YY1 overexpression in liver cells could markedly activate HSCs by inducing IL-1β and IL-18 production, thus activating pyroptosis.

Discussion

TGF-β1 is a member of the TGF-β superfamily and is increased in the development of hepatic fibrosis. CCl_4-induced hepatic injury and TGF-β1-induced HSCs or liver cells are well-established models for hepatic fibrogenesis research (Xu et al., 2016; Yanguas et al., 2016). In the current study, we confirmed the profibrotic effect of YY1 in hepatic fibrosis by using TGF-β1-induced liver cells and a CCl_4-induced hepatic injury rat model, verifying that YY1 could induce HSC activation by facilitating liver cell pyroptosis.

HSC activation plays a crucial role in hepatic fibrogenesis. During the process, HSC activation causes excessive deposition of α-SMA and ECM-associated proteins, like Collagen I and Collagen III, thus accelerating fibrogenesis (He et al., 2020). In our study, we established a hepatic fibrosis rat model through CCl_4 administration. The expression of α-SMA was increased in the liver tissues and primary HSCs isolated from model rats. Meanwhile, we also detected high expression of α-SMA in TGF-β1-induced primary HSCs isolated from normal rats. A high fibrotic level, excessive inflammation, infiltration, and cell apoptosis were found in the liver tissues of CCl_4-induced rats. We successfully established a hepatic fibrosis rat model. Importantly, our data show that with increasing α-SMA and the occurrence of fibrosis, apoptosis, and inflammation, the expression of YY1 also increases in the liver tissue and primary HSCs isolated from model rats and TGF-β1-induced primary HSCs isolated from normal rats.

YY1 is a ubiquitously expressed zinc finger-containing transcription factor. It was reported that YY1 plays a crucial role in the development of a series of disorders, such as cancer, neuropathic pain, and liver disease (Zhang et al., 2017; Sarvagalla et al., 2019; Sun et al., 2021). In a mouse diabetic nephropathy model, YY1 was highly expressed and its expression was associated with kidney fibrosis. Increased YY1 accelerated kidney fibrogenesis by facilitating α-SMA expression and epithelial-mesenchymal transition, which is a key event in fibrogenesis (Yang et al., 2019). In the current study, our data affirmed that fibrosis, inflammation, and cell apoptosis in liver tissues of hepatic fibrosis rats were attenuated following YY1 knockdown. In addition, the expression of α-SMA and pro-inflammatory cytokines (IL-1β and IL-18) in liver tissues of hepatic fibrosis rats was also suppressed by YY1 knockdown. These data suggested that knockdown of YY1 contributed to improving CCl_4-induced hepatic fibrosis in rats.

NLRP3 inflammasomes are large multiprotein complexes, which consist of the inflammasome sensor molecule NLRP3, adaptor protein ASC, and effector molecule pro-caspase-1. Upon stimulation, NLRP3 binds to ASC, resulting in caspase-1 activation. Activated caspase-1 will induce pro-IL-1β and pro-IL-18 maturation and the secretion of IL-1β and IL-18, thus triggering the pro-inflammatory response and activating pyroptosis (Wree et al., 2014; Shi et al., 2017; Du et al., 2021). Moreover, activated caspase-1 cleaves Gasdermin D to generate an N-terminal fragment, GSDMD-N, that forms membrane pores and induces the maturation and secretion of IL-1β and IL-18 (Zhang et al., 2020; Mayes-Hopfinger et al., 2021). Accumulating studies have revealed that pyroptosis plays an important role in hepatic fibrogenesis and exploration of the pathogenesis of pyroptosis in hepatic fibrosis might lead to a novel target for disease treatment (Gan et al., 2022). In our study, expression and secretion of pyroptosis-associated pro-inflammatory cytokines (IL-1β and IL-18) in the liver tissue of hepatic fibrosis rats and TGF-β1-treated liver cells were reduced following the knockdown of YY1. TGF-β1-induced NLRP3, caspase-1, and GSDMD-N overexpression in liver cells were also limited by YY1 knockdown. In summary, YY1 knockdown effectively suppressed liver cell pyroptosis. Subsequently, to probe whether YY1-induced pyroptosis in liver cells affects HSC activation, we collected cell culture medium from liver cells to culture HSCs. Our results revealed that TGF-β1-treated liver cell conditional medium induced HSC activation, which was reflected in the increased proliferation and α-SMA and Collagen III expression in HSCs, while these phenomena were rescued by YY1 knockdown. In addition, increased YY1 promoted IL-1β and IL-18 production in liver cells, which was rescued by the NLRP3 inflammasome inhibitor. The activation of HSCs was enhanced by YY1-overexpressed liver cell conditional medium, however, the activation of HSCs was attenuated when YY1-overexpressed liver cells were treated with NLRP3 inhibitor.

Conclusion

In conclusion, our results revealed that YY1-

overexpressed liver cells facilitated HSC activation by secreting IL-1β and IL-18, which induced NLRP3 inflammasome-mediated pyroptosis, thus aggravating hepatic fibrogenesis. Our data could provide a novel idea for the treatment of hepatic fibrosis and its associated liver disease.

Acknowledgements. We would like to give our sincere gratitude to the reviewers for their constructive comments.

Funding. This work was supported by Key R&D projects of the Jiangxi Provincial Department of Science and Technology (20202BBGL73031).

Ethics approval and consent to participate. Animal protocols were approved by the Animal Care and Use Committee of the Second Affiliated Hospital of Nanchang University. Animal experiments were performed according to the confirmed guidelines.

Consent for publication. The informed consent was obtained from study participants.

Availability of data and material. All data generated or analyzed during this study are included in this published article.

Competing interests. The authors declare that they have no conflict of interest.

References

Dewidar B., Meyer C., Dooley S. and Meindl-Beinker N.. (2019). TGF-β in hepatic stellate cell activation and liver fibrogenesis-updated 2019. Cells 8, 1419.

Du T., Gao J., Li P., Wang Y., Qi Q., Liu X., Li J., Wang C. and Du L. (2021). Pyroptosis, metabolism, and tumor immune microenvironment. Clin. Transl. Med. 11, e492.

Gan C., Cai Q., Tang C. and Gao J. (2022). Inflammasomes and pyroptosis of liver cells in liver fibrosis. Front. Immunol. 13, 896473.

Gaul S., Leszczynska A., Alegre F., Kaufmann B., Johnson C.D., Adams L.A., Wree A., Damm G., Seehofer D., Calvente C.J., Povero D., Kisseleva T., Eguchi A., McGeough M.D., Hoffman H.M., Pelegrin P., Laufs U. and Feldstein A.E. (2021). Hepatocyte pyroptosis and release of inflammasome particles induce stellate cell activation and liver fibrosis. J. Hepatol. 74, 156-167.

Gheorghe G., Bungău S., Ceobanu G., Ilie M., Bacalbaşa N., Bratu O.G., Vesa C.M. and Găman M.-A. (2021). The non-invasive assessment of hepatic fibrosis. J. Formos. Med. Assoc. 120, 794-803.

Gordon S., Akopyan G., Garban H. and Bonavida B. (2006). Transcription factor YY1: structure, function, and therapeutic implications in cancer biology. Oncogene 25, 1125-1142.

He Z., Yang D., Fan X., Zhang M., Li Y. and Gu X. (2020). The Roles and Mechanisms of lncRNAs in Liver Fibrosis. Int. J. Mol. Sci. 21, 1482.

Higashi T., Friedman S.L. and Hoshida Y. (2017). Hepatic stellate cells as key target in liver fibrosis. Adv. Drug Deliv. Rev. 121, 27-42.

Jiang X., Shen T., Tang X., Yang W., Guo H. and Ling W. (2017). Cyanidin-3-O-β-glucoside combined with its metabolite protocatechuic acid attenuated the activation of mice hepatic stellate cells. Food Funct. 8, 2945-2957.

Koh E.H., Yoon J.E., Ko M.S., Leem J., Yun J.-Y., Hong C.H., Cho Y.C., Lee S.E., Jang J.E., Baek J.Y., Yoo H.J., Kim S.J., Sung C.O., Lim J.S., Jeong W.-L., Back S.H., Baek I.-J., Torres S., Solsona-Vilarrasa E., de la Rosa L.C., Garcia-Ruiz C., Feldstein A.E., Fernandez-Checa J.C. and Lee K.-U. (2021). Sphingomyelin synthase 1 mediates hepatocyte pyroptosis to trigger non-alcoholic steatohepatitis. Gut 70, 1954-1964.

Liu H., Zhang S., Xu S., Koroleva M., Small E.M. and Jin Z.G. (2019). Myofibroblast-specific YY1 promotes liver fibrosis. Biochem. Biophys. Res. Commun. 514, 913-918.

Mayes-Hopfinger L., Enache A., Xie J., Huang C.-L., Köchl R., Tybulewicz V.L.J., Fernandes-Alnemri T. and Alnemri E.S. (2021). Chloride sensing by WNK1 regulates NLRP3 inflammasome activation and pyroptosis. Nat. Commun. 12, 4546.

Ou X., Huo J., Huang Y., Li Y.-F., Xu S. and Lam K.-P. (2019). Transcription factor YY1 is essential for iNKT cell development. Cell Mol. Immunol. 16, 547-556.

Roehlen N., Crouchet E. and Baumert T.F. (2020). Liver fibrosis: Mechanistic concepts and therapeutic perspectives. Cells 9, 875.

Sarvagalla S., Kolapalli S.P. and Vallabhapurapu S. (2019). The two sides of YY1 in cancer: A friend and a foe. Front. Oncol. 9, 1230.

Shi J., Gao W. and Shao F. (2017). Pyroptosis: Gasdermin-mediated programmed necrotic cell death. Trends Biochem. Sci. 42, 245-254.

Sun M., Sun Y., Ma J. and Li K. (2021). YY1 promotes SOCS3 expression to inhibit STAT3-mediated neuroinflammation and neuropathic pain. Mol. Med. Rep. 23, 103.

Wree A., Eguchi A., McGeough M.D., Pena C.A., Johnson C.D., Canbay A., Hoffman H.M. and Feldstein A.E. (2014). NLRP3 inflammasome activation results in hepatocyte pyroptosis, liver inflammation, and fibrosis in mice. Hepatology 59, 898-910.

Xu F., Liu C., Zhou D. and Zhang L. (2016). TGF-β/SMAD pathway and Its regulation in hepatic fibrosis. J. Histochem. Cytochem. 64, 157-167.

Yang T., Shu F., Yang H., Heng C., Zhou Y., Chen Y., Qian X., Du L., Zhu X., Lu Q. and Yin X. (2019). YY1: A novel therapeutic target for diabetic nephropathy orchestrated renal fibrosis. Metabolism 96, 33-45.

Yang F., Li H., Li Y., Hao Y., Wang C., Jia P., Chen X., Ma. And Xiao Z. (2021). Crosstalk between hepatic stellate cells and surrounding cells in hepatic fibrosis. Int. Immunopharmacol. 99, 108051.

Yanguas S.C., Cogliati B., Willebrords J., Maes M., Colle I., van der Bossche B., de Oliveira C.P.M.S., Andraus W., Alves V.A.F., Leclercq I. and Vinken M. (2016). Experimental models of liver fibrosis. Arch. Toxicol. 90, 1025-1048.

Yu P., Zhang X., Liu N., Tang L., Peng C. and Chen X. (2021). Pyroptosis: mechanisms and diseases. Signal. Transduct. Target. Ther. 6, 128.

Zhang M., Zhang Y., Yang S., Zhou J., Gao W., Yang X., Yang D., Tian Z., Wu Y. and Ni B. (2017). Multifunctional YY1 in liver liseases. Semin. Liver Dis. 37, 363-376.

Zhang C., Zhu X., Hua Y., Zhao Q., Wang K., Zhen L., Wang G., Lü J., Luo A., Cho W.C., Lin X. and Yu Z. (2019). YY1 mediates TGF-β1-induced EMT and pro-fibrogenesis in alveolar epithelial cells. Respir. Res. 20, 249.

Zhang K., Shi Z., Zhang M., Dong X., Zheng L., Li G., Han X., Yao Z. Han T. and Hong W. (2020). Silencing lncRNA Lfar1 alleviates the classical activation and pyoptosis of macrophage in hepatic fibrosis. Cell Death Dis. 11, 132.

Accepted January 4, 2024