Biochimica et Biophysica Acta 1842 (2014) 2367–2377

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

GATA binding protein 2 mediates leptin inhibition of PPARγ1 expression in hepatic stellate cells and contributes to hepatic stellate cell activation

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ARTICLE INFO

Article history:
Received 28 June 2014
Received in revised form 15 September 2014
Accepted 1 October 2014
Available online 8 October 2014

Keywords:
Leptin
GATA binding protein 2
Peroxisome-proliferator activated receptor γ1
Liver fibrosis
Hepatic stellate cell

ABSTRACT

Hepatic stellate cell (HSC) activation is a crucial step in the development of liver fibrosis. Peroxisome-proliferator activated receptor γ (PPARγ) exerts a key role in the inhibition of HSC activation. Leptin reduces PPARγ expression in HSCs and plays a unique role in promoting liver fibrosis. The present studies aimed to investigate the mechanisms underlying leptin regulation of PPARγ1 (a major subtype of PPARγ) in HSCs in vivo and in vitro. Results revealed a leptin response region in mouse PPARγ1 promoter and indicated that the region included a GATA binding protein binding site around position −2323. GATA binding protein-2 (GATA-2) could bind to the site and inhibit PPARγ1 promoter activity in HSCs. Leptin induced GATA-2 expression in HSCs in vitro and in vivo. GATA-2 mediated leptin inhibition of PPARγ1 expression by its binding site in PPARγ1 promoter in HSCs and GATA-2 promoted HSC activation. Leptin upregulated GATA-2 expression through β-catenin and sonic hedgehog pathways in HSCs. Leptin-induced increase in GATA-2 was accompanied by the decrease in PPARγ expression in HSCs and by the increase in the activated HSC number and liver fibrosis in vivo. Our data might suggest a possible new explanation for the promotion effect of leptin on liver fibrogenesis.

1. Introduction

Liver fibrosis is the result of chronic liver injury from multiple causes. The fibrogenic response is characterized by progressive accumulation of extracellular matrix (ECM) which is produced mainly by activated hepatic stellate cell (HSC) [1]. HSC activation is a crucial step in the development of liver fibrosis [1]. The process of HSC activation is the transdifferentiation from quiescent to myofibroblastic cell and requires global reprogramming of HSC gene expression which must be orchestrated by key transcription regulators. Quiescent HSCs are much like adipocyte and HSC activation appears to be analogous to adipocyte to preadipocyte transdifferentiation [2]. It has been shown that peroxisome-proliferator activated receptor γ (PPARγ) and sterol regulatory element binding protein-1c (SREBP-1c), the transcription factors (TFs) controlling adipocyte differentiation, play key roles in the inhibition of HSC activation [2,3]. Overexpression of PPARγ or SREBP-1c leads to morphologic and biochemical reversals of activated HSCs to quiescent cells [4].

Liver fibrosis is shown to be six times more prevalent in obese patients as compared with general population [5,6] while obese patients are often accompanied by hyperleptinemia [7,8]. Accumulating evidence demonstrates that leptin, an adipocyte-derived hormone, plays a unique role in promoting liver fibrosis in vitro and in vivo [9–13]. Therefore, we observed the effect of leptin on PPARγ in HSCs in vitro and in vivo and showed that leptin evidently reduced PPARγ expression in HSCs, hence contributing to HSC activation [14,15]. The underlying mechanisms for leptin inhibition of PPARγ expression in HSCs have not been fully elucidated. PPARγ include two subtypes, namely PPARγ1 and PPARγ2, and PPARγ1 is the major subtype of PPARγ in HSCs [16]. Thus, the present studies were aimed to investigate the mechanisms underlying leptin regulation of PPARγ1 in HSCs in vitro and in vivo, mainly focusing on: 1) TFs mediating the effect of leptin on PPARγ1 expression in HSCs; and 2) the signaling pathways by which leptin regulates the correlated TFs.

2. Materials and methods

2.1. Materials

Leptin was purchased from ProSpec-Tany TechnoGene (Rehovot, Israel) and used to treat HSCs in vitro at 100 ng/ml [13], unless otherwise stated. XAV939 (a specific inhibitor for β-catenin pathway) and cyclopamine (a specific inhibitor for Hedgehog (Hh) signaling pathway) were purchased from Santa Cruz (CA, USA) and selleck Chemicals

Abbreviations: HSC, Hepatic stellate cell; PPARγ1, Peroxisome-proliferator activated receptor γ1; GATA -2, GATA binding protein 2; Shh, Sonic hedgehog; ECM, Extracellular matrix; TAA, Thioacetamide; FBS, Fetal bovine serum; DMEM, Dulbecco's modified Eagle's medium; α-SMA, Alpha-smooth muscle actin

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http://dx.doi.org/10.1016/j.bbadis.2014.10.001
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(Houston, USA), respectively. Thioacetamide (TAA) was from Sigma (St. Louis, MO, USA). Sonic Hedgehog N-terminus (ShhN) was from R&D Systems (Minneapolis, MN, USA).

2.2. HSC isolation and culture

HSCs were isolated from adult Kunming mice (Animal Research Center of Nantong University, Nantong, China) as we described previously [15]. HSCs between passages 3 and 6 were used for experiments. After 12 h of serum starvation in Dulbecco's modified Eagle's medium (DMEM) with 1 % fetal bovine serum (FBS), HSCs were treated with leptin, unless otherwise stated.

2.3. Animal studies

C57BL/6J ob/ob mice (leptin-deficient obese mouse, Model Animal Research Center of Nanjing University, Nanjing, China), 6 week old, were randomly separated into two groups (six mice/each group). We adopted a mouse model of TAA-induced liver damage [17]. TAA is usually used for induction of mouse liver injury [13,17].

The first two groups were given the administration of TAA (200 μg/g body weight, two times a week) or TAA plus leptin (1 μg/g body weight, once per day) by intraperitoneal injection (i.p.) for 4-week [13,17].

The second two groups were treated with Ad.Fc (a control adenovirus encoding IgG2α Fc fragment, a gift from Dr. Jill A. Helms, Stanford University, USA) or Ad.Dkk1 (adenovirus encoding mouse Dickkopf-1, 2×10^7 pfu/g body weight, once every two weeks, a gift from Dr. Jill A. Helms) [18] by tail vein throughout the 4-week period of TAA plus leptin treatment as previously described [13].

The third two groups were treated with cyclopamine (1 μg/g body weight, once per day) or vehicle by i.p. throughout the 4-week period of TAA plus leptin treatment.

After 4-week, the livers were fixed in 4% buffered paraformaldehyde for immunostaining analysis or livers were used for Western blot analysis and hydroxyproline determination or HSCs were isolated from mice for Western blot analysis. All the mice were given free access to water and standard chow diet and received humane care. Experiments were approved by the Institutional Animal Care and Use Committee of the University of Nantong (2012-0031).

2.4. Immunofluorescence staining and sirius red staining

Double fluorescence staining was used to examine the expression of Sonic hedgehog (Shh), β-catenin, GATA binding protein 2 (GATA-2), and PPARγ in HSCs in the liver as we described previously [13]. Briefly, paraformaldehyde-fixed liver sections were blocked with normal serum and incubated with primary antibody against Shh (1:50, Santa Cruz, CA, USA), β-catenin (1:100, Santa Cruz, CA, USA), GATA-2 (1:50, Santa Cruz, CA, USA), PPARγ (1:100, Abcam, MA, USA) and primary antibody against synaptophysin (SYP, 1:10, Abcam, MA, USA), a marker for quiescent and activated HSCs [19], followed by incubation with DyLight594-conjugated secondary antibody (1:500, ImmunoReagents, Inc., Raleigh, USA) and DyLight488-conjugated secondary antibody (1:500, ImmunoReagents, Inc., Raleigh, USA). For single fluorescence staining of α-smooth muscle actin (α-SMA) on the liver sections, paraformaldehyde-fixed liver sections were blocked with normal serum and incubated with primary antibody against α-SMA (1:100, Abcam) and subsequently the DyLight594-conjugated secondary antibody. The nuclei were counterstained with Hoechst 33342 (Sigma, St. Louis, MO, USA). The images were captured with the fluorescence microscope and representative images were shown.

For the analysis of liver fibrosis, sirius red was used to stain collagen on liver sections. Briefly, paraformaldehyde-fixed liver sections were stained with picric acid-fast green (Amresco, Solon, USA) and then incubated with picric acid–sirius red (Amresco, Solon, USA) for 1 h. Images were captured with light microscope.

2.5. Plasmid constructs and transient transfection assay

To construct PPARγ1 promoter luciferase reporter plasmid, the mouse PPARγ1 promoter (from −2333 to +157) was amplified from genomic DNA of Kunming mice and was inserted into KpnI/XhoI sites of pGL3-basic (Promega, Madison, USA). This constructed plasmid was named as pPPARγ1(−2333)Luc. The short PPARγ1 promoter reporter plasmids were constructed by using pPPARγ1(−2333)Luc and were named as pPPARγ1 (−1823)Luc (from −1823 to +157), pPPARγ1 (−2245)Luc (from −2245 to +157).

To construct the site-mutated PPARγ1 promoter reporter plasmids, the potential GATA-2 binding site in PPARγ1 (around the site of −2323) was mutated by using pPPARγ1(−2333)Luc and KOD-Plus-Mutagenesis Kit (TOYOBO CO., LTD., Osaka, Japan) according to the manufacturer's instructions. The plasmid was named as pPPARγ1(GATA mut) Luc.

To construct luciferase reporter plasmid containing three tandem repeats of potential GATA-2 binding site of PPARγ1 promoter, tandem repeats of potential GATA-2 binding site were synthesized and cloned into KpnI/XhoI site upstream of SV40 promoter in pGL3-promoter vector (Promega, Madison, USA). The plasmid was named as p3xGATA-Luc and the sense of strand DNA was 3 × (5′-tcttttGATAtgtgcaga-3′).

For constructing mouse GATA-2 promoter luciferase reporter plasmid, GATA-2 promoter (from −2657 to +118) was amplified from genomic DNA of Kunming mice and was inserted into MluI/XhoI sites of pGL3-basic. The plasmid was named as pGATA2(−2657)Luc.

For constructing plasmid encoding GATA-2 protein, total RNA was extracted from mouse cultured HSCs by using TRI-Reagent (Sigma, St. Louis, USA) and reverse transcripted into cDNA. Then, GATA-2 cDNA was amplified from total cDNA and cloned into KpnI/XhoI sites of pcDNA3.1 (Invitrogen, CA, USA). The plasmid was named as pcDNA-GATA2.

All the primers were used for the construction of the respective plasmid and for mutation were shown in supplemental data 1. All the sequences of the constructed plasmids were confirmed by DNA sequence analysis.

Plasmid piLentsiRNA-GATA2 (encoding small interfering RNA targeting mouse GATA-2) and piLentsiRNA-GFP (control) were from abm (Richmond, BC, Canada).

HSCs in twelve-well plastic plates (unless otherwise stated) were transiently transfected with the respective plasmid with by using LipofectAMINE reagent (Life Technologies, New York, USA) according to manufacturer's instructions. In cells transfected with reporter plasmid, 30 ng of control vector expressing Renilla luciferase (pRL-TK; Promega, Madison, USA) was cotransfected into the cells. Luciferase activity was quantified fluorimetrically by using the Dual-Luciferase Reporter Assay System (Promega, Madison, USA) and the data were expressed as the ratios of Photinus to Renilla luciferase activity for normalization of Photinus luciferase activity.

2.6. Western blot analysis

Western blot analyses were conducted as we described previously [15]. Briefly, target protein was detected by primary antibody against GATA-2 (1:500, Santa Cruz), PPARγ (1:500, Abcam), α1(I)collagen (1:2000, Santa Cruz), α-SMA (1:2000, Abcam), Shh (1:500, Santa Cruz), β-Catenin (1:500, Santa Cruz), transforming growth factor β1 (TGFβ1,1: 500, Santa Cruz), platelet-derived growth factor receptor β(PDGFRβ, 1:1000), matrix metalloproteinases 1 (MMP1, 1:500, Abcam), matrix metalloproteinases 2 (MMP2, 1:500, Abcam), tissue inhibitor of metalloproteinases 1 (TIMP1, 1:500, Santa Cruz), tissue inhibitor of metalloproteinases 2 (TIMP2, 1:500, Santa Cruz), or β-actin (1:2000, Santa Cruz) and subsequently by horseradish peroxidase-conjugated secondary antibody (1:4000). β-Actin was used as an internal control.

2.7. RNA isolation and real-time PCR

Total RNA was extracted by using TRI-Reagent (Sigma, St. Louis, USA) according to the manufacturer's instructions. Real-time PCR was performed as we described previously [14]. For the analysis of fold change in mRNA level of target gene relative to the endogenous cyclophilin control, the Ct values were normalized against cyclophilin and analyzed by using the ΔΔCt method. The primers for real-time PCR were shown in supplemental data 1.

2.8. Electrophoretic mobility shift assay (EMSA)

EMSA assays were performed by using LightShift Chemiluminescent EMSA Kit (Pierce Biotechnology, IL, USA). Briefly, nuclear proteins were firstly extracted by NE-PER Nuclear and Cytoplasmic Extraction Reagents according to the manufacturer's instructions (Pierce Biotechnology, Rockford, IL, USA). Biotinylated DNA fragments (labeled probes) between −2333 and −2304 (containing the potential GATA-2 binding site, shown in supplemental data 1) of mouse PPARγ1 promoter was synthesized by Life Technologies (Shanghai, China) and used for GATA-2 binding assay. The labeled probes were incubated with 5 μg protein of nuclear extract in the binding buffer at 25 °C for 20 min. For the competition assay, 5 μg protein of nuclear extract was preincubated with 100-fold molar excess of the unlabeled probes before the addition of the labeled probe. For supershift assay, 5 μg protein of nuclear extract was preincubated with 1 μg of GATA-2 antibody before the addition of the labeled probes. The samples were subjected to electrophoresis in a 5% nondenaturating polyacrylamide gel and transferred onto a nylon membrane and detected by Substrate Working Solution.

2.9. Chromatin immunoprecipitation (ChIP)

ChIP assays were carried out as we described previously [20] by using Pierce Agarose Chip Kit (Pierce, Rockford, IL, USA). Briefly, cultured HSCs were cross-linked with 1% formaldehyde and the nuclei from the cells were incubated with Micrococcal Nuclease and lysed. 10% of the digested chromatin was preserved as input control and the rest of the digested chromatin were incubated with GATA-2 antibody (Santa Cruz, CA, USA). The purified DNA from immunoprecipitation and the input samples were used for the analysis of a fragment (132 bp) between nucleotides −2362 and −2230 (containing a potential GATA-2 binding site around −2323) by real-time PCR. The used primers were shown in supplementary data 1.

According to the method as described by Mastrogiannaki et al. [21], the amplification by real-time PCR was quantified as the ratio: [2^(Ct input–Ct ChIP) $_{\text{treatment}}$]/[2^(Ct input–Ct ChIP) $_{\text{without treatment}}$], where Ct ChIP is the Ct value corresponding to the immunoprecipitated DNA, and Ct input is the Ct value of an aliquot of digested chromatin sample before immunoprecipitation.

2.10. Hydroxyproline determination

Hydroxyproline was determined biochemically as described [22] and the HP content was expressed as μg/g of wet liver.

2.11. Statistical analysis

The results are expressed as mean values ± standard deviation (S.D.). Differences between means were evaluated using an unpaired two-sided Student's *t*-test. Where appropriate, comparisons of multiple treatment conditions with controls were analyzed by ANOVA with the Dunnett's test for post hoc analysis. Each result was obtained from at least three independent differentiation experiments. *P* value < 0.05 is considered as significant.

3. Results

3.1. Leptin response elements exist between position −2333 and −2245 upstream of the transcription start site of PPARγ1 promoter

To identify leptin response elements in PPARγ1 promoter, HSCs transfected with pPPARγ1(-2333)Luc or pPPARγ1 (−1823)Luc were treated with or without leptin. Fig. 1A showed that luciferase activity was reduced in cells with pPPARγ1(−2333)Luc but not pPPARγ1 (−1823) by leptin, suggesting a DNA fragment between −2333 and −1823 in PPARγ1 promoter contained leptin response elements. Next, pPPARγ1 (−2245) Luc was used to transfected HSCs and treated with or without leptin. Fig. 1B showed that leptin failed to affect the luciferase activity, suggesting that leptin response elements existed between position −2333 and −2245 in PPARγ1 promoter.

The possible TF binding sites in the DNA fragment (between −2333 and −2245) of PPARγ1 promoter were predicted by software BioBase (http://www.biobase-international.com/). Some of the possible TF binding sites including the potential GATA protein binding site (around position −2323 upstream of the transcription start site) were underlined (supplemental data 2). PPARγ plays key roles in promoting adipocyte differentiation [23] and in inhibiting HSC activation [2]. In contrast, GATA proteins serve as the negative regulators of adipocyte formation [24,25]. Since leptin treatment reduced PPARγ expression in HSCs [14,15], it was interesting to investigate whether GATA proteins bound to the potential GATA protein binding site in PPARγ1 promoter and mediated leptin inhibition of PPARγ1 expression in HSCs. As transcriptional factor GATA-2, a member of the GATA protein, is a gatekeeper by controlling the transition from preadipocytes to adipocytes [26], we firstly hypothesized that GATA-2 might mediate leptin inhibition of PPARγ1 expression by binding to the potential site in PPARγ1 promoter in HSCs.

3.2. GATA-2 inhibits PPARγ1 promoter activity by binding to a site around position -2323 of PPARγ1 promoter and reduces PPARγ1 expression

To test whether the possible GATA protein binding site (around position of −2323) affected PPARγ1 promoter activity, the site was mutated by using pPPARγ1(−2333)Luc and the constructed plasmid is named as pPPARγ1(GATA mut) Luc. HSCs was transfected with pPPARγ1(−2333)Luc (control) or pPPARγ1(GATA mut) Luc and incubated with leptin. Fig. 2A demonstrated that the mutation of the possible GATA protein binding site led to the increase in luciferase activity. This result revealed the inhibitory effect of the site on PPARγ1 promoter activity in the presence of leptin in HSCs.

For the evaluation of the effects of GATA-2 on PPARγ1 promoter activity and transcription activity, HSCs were cotransfected with pPPARγ1(−2333)Luc plus pcDNA-GATA2 (encoding GATA-2) or vector

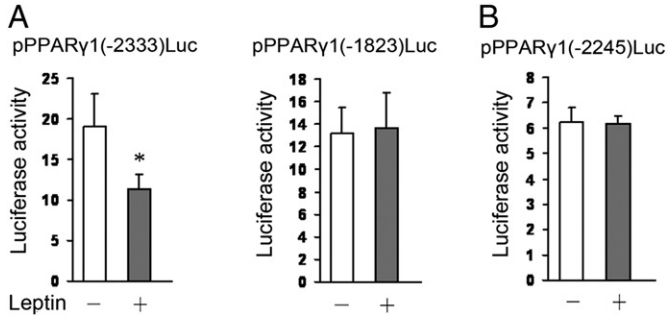

Fig. 1. Leptin response elements exist between position −2333 and −2245 upstream of the transcription start site of PPARγ1 promoter. HSCs were transfected with 1.6 μg of the respective reporter plasmid containing PPARγ1 promoter of different lengths and treated with or without leptin for 24 h. Luciferase assays were performed (n = 3). $^{*}P < 0.05$ vs the control without leptin.

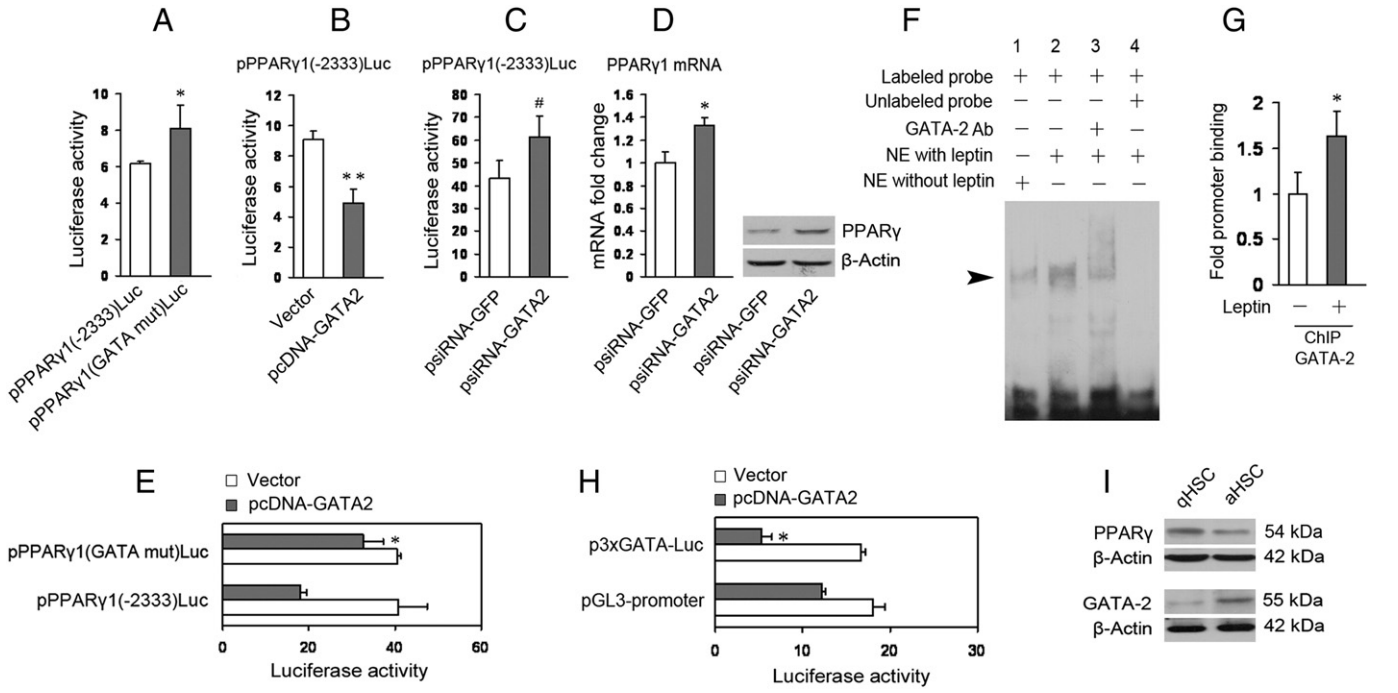

Fig. 2. GATA-2 reduces PPARγ1 expression and activity by binding to PPARγ1 promoter. (A, B, C) Transfection assay for analysis of PPARγ1 promoter activity (n = 3). The first group of HSCs was transfected with 1.6 μg of pPPARγ1(-2333)Luc or pPPARγ1(GATA mut) Luc and incubated with leptin for 24 h (A). The second group of HSCs was cotransfected with 0.8 μg of pPPARγ1(-2333)Luc and 0.8 μg of pcDNA-GATA2 (encoding GATA-2) or control vector and incubated for 24 h (B). The third group of HSCs was cotransfected with 0.8 μg of pPPARγ1(-2333)Luc plus 0.8 μg of piLentsiRNA-GATA2 or piLentsiRNA-GFP (control) and incubated with leptin for 48 h (C). Luciferase assays were performed. $^{*}P < 0.05$ vs the cells with pPPARγ1(−2333)Luc. $^{**}P < 0.05$ vs the cells with vector. $^{\#}P < 0.05$ vs the cells with piLentsiRNA-GFP. (D) Real-time PCR of PPARγ1 expression and Western blot analyses of PPARγ expression (n = 3). HSCs in six-well plastic plates were transfected with 3.2 μg of piLentsiRNA-GATA2 or piLentsiRNA-GFP and incubated with leptin for 48 h, PPARγ1 mRNA levels and PPARγ protein levels were determined respectively by real-time PCR and Western blot analyses. β-Actin was used as an internal control. $^{*}P < 0.05$ vs the cells with piLentsiRNA-GFP. (E) Transfection assay for analysis of PPARγ1 promoter activity (n = 3). HSCs was cotransfected with 0.8 μg of pPPARγ1(-2333)Luc or pPPARγ1(GATA mut) Luc plus 0.8 μg of pcDNA-GATA2 or vector and incubated for 24 h. Luciferase assays were performed. $^{*}P < 0.05$ vs the cells with pPPARγ1(−2333)Luc plus pcDNA-GATA2. (F) EMSA of the interaction between GATA-2 and a possible GATA-2 binding site (around position −2323) of PPARγ1 promoter. HSCs were stimulated with or without leptin for 24 h and nuclear extracts (NE) were prepared. 5 μg of nuclear proteins were incubated with biotinylated DNA fragment (labeled probe containing the potential GATA-2 binding site around −2323). For competition assay, 5 μg of nuclear proteins from cells treated with leptin was preincubated with 100-fold molar excess of the unlabeled probe before the addition of labeled probe. For supershift assay, 5 μg of nuclear proteins from cells treated with leptin was preincubated with 1 μg of anti-GATA2 antibody before the addition of the labeled probe. A representative EMSA was shown from three independent experiments. (G) ChIP analysis of the interaction between GATA-2 and the possible GATA-2 binding site (around −2323) of PPARγ1 promoter (n = 3). HSCs were stimulated with or without leptin for 24 h and ChIP analysis was performed by using anti-GATA2 antibody. A fragment (132 bp) between nucleotides −2362 and −2230 (containing the possible GATA-2 binding site) in PPARγ1 promoter was examined by real-time PCR. $^{*}P < 0.05$ vs the cells without leptin. (H) Transfection assay for the analysis of the activity of an artificial promoter harboring three tandem repeats of the possible GATA-2 binding site in PPARγ1 (n = 3). HSCs were cotransfected with 0.8 μg of p3xGATA-Luc or pGL3-promoter vector (control) plus 0.8 μg of pcDNA-GATA2 or vector and incubated for 24 h. Luciferase assay was performed. $^{*}P < 0.05$ vs the cells with pGL3 promoter plus pcDNA-GATA2. (I) Western blot analyses of the expressions of PPARγ and GATA-2 (n = 3). HSCs isolated from the normal mouse liver (quiescent HSCs,qHSCs) or HSCs cultured for 7 days on a plastic dish (activated HSCs,aHSCs) were used for Western blot analyses. β-Actin was used as an internal control.

and the result showed that pcDNA-GATA2 significantly reduced PPARγ1 promoter activity (Fig. 2B). Next, HSCs were cotransfected with pPPARγ1(−2333)Luc plus piLentsiRNA-GATA2 (for knockdown of GATA-2 expression) or piLentsiRNA-GFP (control) (Fig. 2C) or HSCs were transfected with piLentsiRNA-GATA2 or piLentsiRNA-GFP (Fig. 2D). After all the transfected cells were incubated with leptin for 48 h, the luciferase activities (Fig. 2C), PPARγ1 mRNA levels (Fig. 2D), and PPARγ protein levels (PPARγ1 is the major subtype of PPARγ in HSCs [16] and PPARγ1 antibody is not available, thus PPARγ was detected by using PPARγ antibody) (Fig. 2D) were examined. Results indicated that the knockdown of GATA-2 expression led to the increases in luciferase activity (Fig. 2C), PPARγ1 mRNA level (Fig. 2D), and PPARγ protein levels (Fig. 2D). Results in Fig. 2B–D pointed to the inhibitory effect of GATA-2 on PPARγ1 expression in HSCs.

To test whether GATA-2 functioned by the potential GATA protein binding site (around −2323), we cotransfected HSCs with pPPARγ1(−2333)Luc or pPPARγ1(GATA mut) Luc plus pcDNA-GATA2 or vector. Fig. 2E showed that the inhibitory effect of pcDNA-GATA2 on the luciferase activity in cells with pPPARγ1(GATA mut) Luc was reduced as compared with that in cells with pPPARγ1(−2333)Luc. These results suggested that GATA-2 might bind to the site (around −2323) in PPARγ1 promoter, thus exerting its inhibitory effect on PPARγ1 expression.

For detecting the direct interaction of GATA-2 with the possible GATA-2 binding site, nuclear proteins were extracted from HSCs stimulated with or without leptin and used for EMSA assay. The possible GATA-2 binding site was used as the probe. Supershift assay (Fig. 2F, lane 3) and competition assay (Fig. 2F, lane 4) demonstrated that the site was bound by GATA-2 protein. As compared with the lane 1 (without leptin treatment), lane 2 (with leptin treatment) demonstrated the increase in the binding of protein to the probe, suggesting that leptin might promote the binding of GATA-2 to the probe. For in vivo examination of the binding of GATA-2 to the probe, HSCs were stimulated with or without leptin and ChIP assay was performed by using GATA-2 antibody (Fig. 2G). A DNA fragment (132 base pairs) between nucleotides −2362 and −2230 (containing the potential GATA-2 binding site) in PPARγ1 promoter was examined by real-time PCR and results showed that leptin increased GATA-2 binding to the DNA fragment (Fig. 2G). EMSA and ChIP assays suggested the binding of GATA-2 to the possible GATA protein binding site around position −2323 in PPARγ1 promoter.

We also constructed a plasmid p3xGATA-Luc containing three tandem repeats of the potential GATA-2 binding site of PPARγ1 and examined the effect of GATA-2 on the artificial promoter. HSCs were cotransfected with p3xGATA-Luc or pGL3-promoter vector (control) plus pcDNA-GATA2 or vector (control) (Fig. 2H). Fig. 2H demonstrated

that the luciferase activity in cells with p3xGATA-Luc plus pcDNA-GATA2 significantly decreased as compared with that with p3xGATA-Luc plus vector, which suggested the GATA-2-induced inhibition of the artificial promoter activity. This result further confirmed that GATA-2 bound to the site (around −2323) in PPARγ1, thus inhibiting PPARγ1 promoter activity.

We also examined the expressions of PPARγ and GATA-2 in quiescent HSCs (isolated from normal mouse liver and directly used for Western blot analysis) and activated HSCs (cultured for 7 days on plastic dish). Western blot analysis showed that, as expected, PPARγ protein level significantly decreased in activated HSCs as compared with that in quiescent HSCs (Fig. 2I), on the contrary, GATA-2 protein level increased in activated HSCs as compared with that in quiescent HSCs (Fig. 2I). These results suggested that the upregulation of GATA-2 was accompanied with the decline in PPARγ expression during the process of HSC activation, which was in line with the effect of GATA-2 on PPARγ expression in HSCs as shown in Fig. 2A–H.

3.3. Leptin induces GATA-2 expression in HSCs in vitro

Since leptin promoted the binding of GATA-2 to PPARγ1 promoter (Fig. 2F), we examined the effect of leptin on GATA-2 expression in HSCs. Fig. 3A indicated that leptin stimulation caused a dose-dependent increase in GATA-2 protein level. Based on the result, HSCs were incubated with or without 100 ng/ml of leptin and GATA-2 mRNA levels were detected. Fig. 3B showed the promotion effect of leptin on GATA-2 expression at transcriptional level. Furtherly, HSCs transfected with pGATA2(-2657)Luc (containing mouse GATA-2 promoter) were incubated with leptin. Luciferase assay indicated that leptin stimulation led to the increase in the GATA-2 promoter activity (Fig. 3C), which was in line with the results in Fig. 3A and B. In view of the fact that GATA-2 bound to the artificial promoter (containing the three tandem repeats of GATA-2 binding site) and inhibited its activity (Fig. 2H), we also detected the effect of leptin on GATA-2 trans-regulatory activity by using p3xGATA-Luc. HSCs were transfected with p3xGATA-Luc and then treated with or without leptin (Fig. 3D). Luciferase assay demonstrated that leptin reduced the luciferase activity in cells with p3xGATA-Luc, suggesting that leptin increased GATA-2 trans-regulatory activity in HSCs.

Taken together, results in Fig. 3A–D strongly suggested the promotion effect of leptin on GATA-2 expression and activity in HSCs in vitro and, combined with the data in Fig. 2, implied that GATA-2 was a mediator for leptin inhibition of PPARγ1 expression in cultured HSCs.

Leptin has been shown to promote the expressions of α-SMA (a well-established marker for HSC activation) and α1(I) collagen (the major component of ECM) in HSCs [11,13] whereas GATA-2 was a mediator for leptin inhibition of PPARγ1, a key TF in the inhibition of HSC activation, in cultured HSCs. Thereby, we investigated the role of GATA-2 in leptin promotion of the expressions of α-SMA and α1(I) collagen in cultured HSCs. HSCs were transfected with

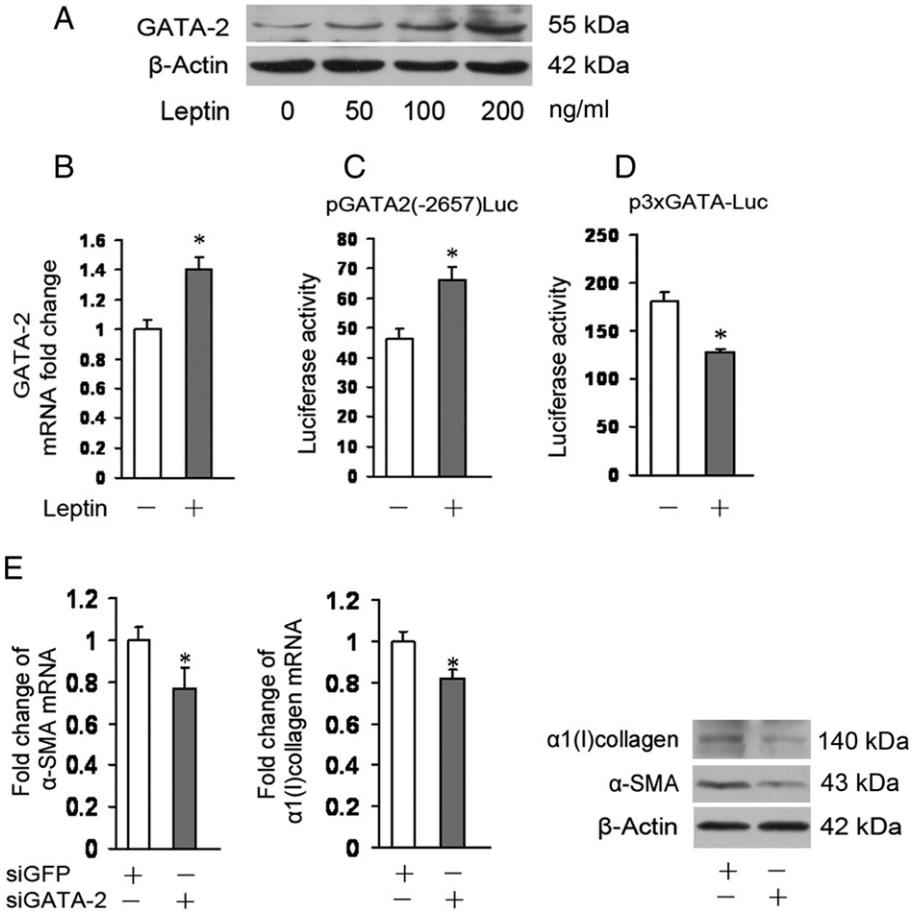

Fig. 3. Leptin stimulates GATA-2 expression and activity. (A, B) Western blot analysis or real-time PCR analysis of GATA-2 expression (n = 3). HSCs were incubated with different doses of leptin for 24 h. GATA-2 protein levels and mRNA levels were examined by western blot analysis and real-time PCR analysis, respectively. Representative blots were shown and β-actin was used as an internal control. *$P < 0.05$ vs the cells without leptin. (C, D) Transfection assay for analysis of GATA2 promoter activity and trans-regulatory activity (n = 3). HSCs were transfected with 1.6 μg of pGATA2(−2657)Luc (C) or p3xGATA-Luc (D) and incubated with or without leptin for 24 h. Luciferase activities were examined. *$P < 0.05$ vs the cells without leptin. (E) Real-time PCR and Western blot analyses of α-SMA and α1(I)collagen expressions (n = 3). HSCs in six-well plastic plates were transfected with 3.2 μg of piLentsiRNA-GATA2 (siGATA-2) or piLentsiRNA-GFP (siGFP, control) and incubated with leptin for 48 h. Real-time PCR and Western blot analyses were performed. *$P < 0.05$ vs the cells with the respective control.

piLentsiRNA-GATA-2 or piLentsiRNA-GFP and incubated with leptin. Real-time PCR and Western blot analyses (Fig. 3E) demonstrated that the knockdown of GATA-2 reduced mRNA and protein levels of α-SMA and α1(I)collagen, which was in line with the role of GATA-2 in leptin inhibition of PPARγ1 in HSCs.

3.4. β-Catenin pathway and Hh pathway are involved in leptin-induced increases in GATA-2 expression and activity in cultured HSCs

Both β-catenin pathway and Hh pathway were demonstrated to mediate the promotion effect of leptin on HSC activation [11,13] and leptin induced the expression of GATA-2 which contributed to HSC activation (Fig. 3). Therefore, there exists a possibility that both β-catenin pathway and Hh pathway might be involved in leptin promotion of GATA-2 expression in HSCs. To address the possibility, HSCs were pretreated with or without 5 μM of XAV939 (inhibiting β-catenin pathway) [13] or 5 μM of cyclopamine (inhibiting Hh pathway) [11] before leptin stimulation. Western blot and real-time PCR analyses (Fig. 4A and B) showed that the interruption of leptin-induced β-catenin pathway or Hh pathway (leptin + XAV or Cyclo) reduced leptin-induced upregulation of GATA-2 protein and mRNA levels, as compared with the respective control with leptin alone. Next, Ad.Dkk1 (encoding Dickkopf-1 inhibiting β-catenin pathway) and ShhN (stimulating Shh pathway) were used to inhibit and stimulate the respective signaling pathway. As shown in Fig. 4C, HSCs infected with Ad.Dkk1 or Ad.Fc (control) were incubated with leptin or HSCs were incubated with ShhN or the vehicle. Real-time PCR and Western blot analyses demonstrated that the inhibition of β-catenin pathway by Ad.Dkk1 reduced the levels of GATA-2 mRNA and protein while the stimulation of Shh pathway increased the levels of GATA-2 mRNA and protein (Fig. 4C), which supported the results in Fig. 4B.

For examining the effect of leptin-induced β-catenin or Hh pathway on GATA-2 promoter activity, the first group of HSCs transfected with pGATA2(-2657)Luc were preincubated with 5 μM of XAV939 or 5 μM of cyclopamine before leptin stimulation. The second group of HSCs was transducted with pGATA2(-2657)Luc and Ad.Dkk1 or the control Ad.Fc and then treated with leptin. The third group of HSCs was transfected with pGATA2(-2657)Luc and incubated with ShhN or the vehicle. Luciferase assays indicated that the interruption of leptin-induced the two signaling pathways by respective inhibitor (Leptin + XAV or Leptin + Cyclo) reduced leptin-induced luciferase activity, as compared with cells treated with leptin only (left panel in Fig. 4D), suggesting that the two pathways induced by leptin increased GATA-2 promoter activity. Moreover, the inhibition of β-catenin signaling pathway by Ad.Dkk1 also reduced the luciferase activity (middle panel in Fig. 4D) and stimulation of Shh signaling pathway by ShhN promoted

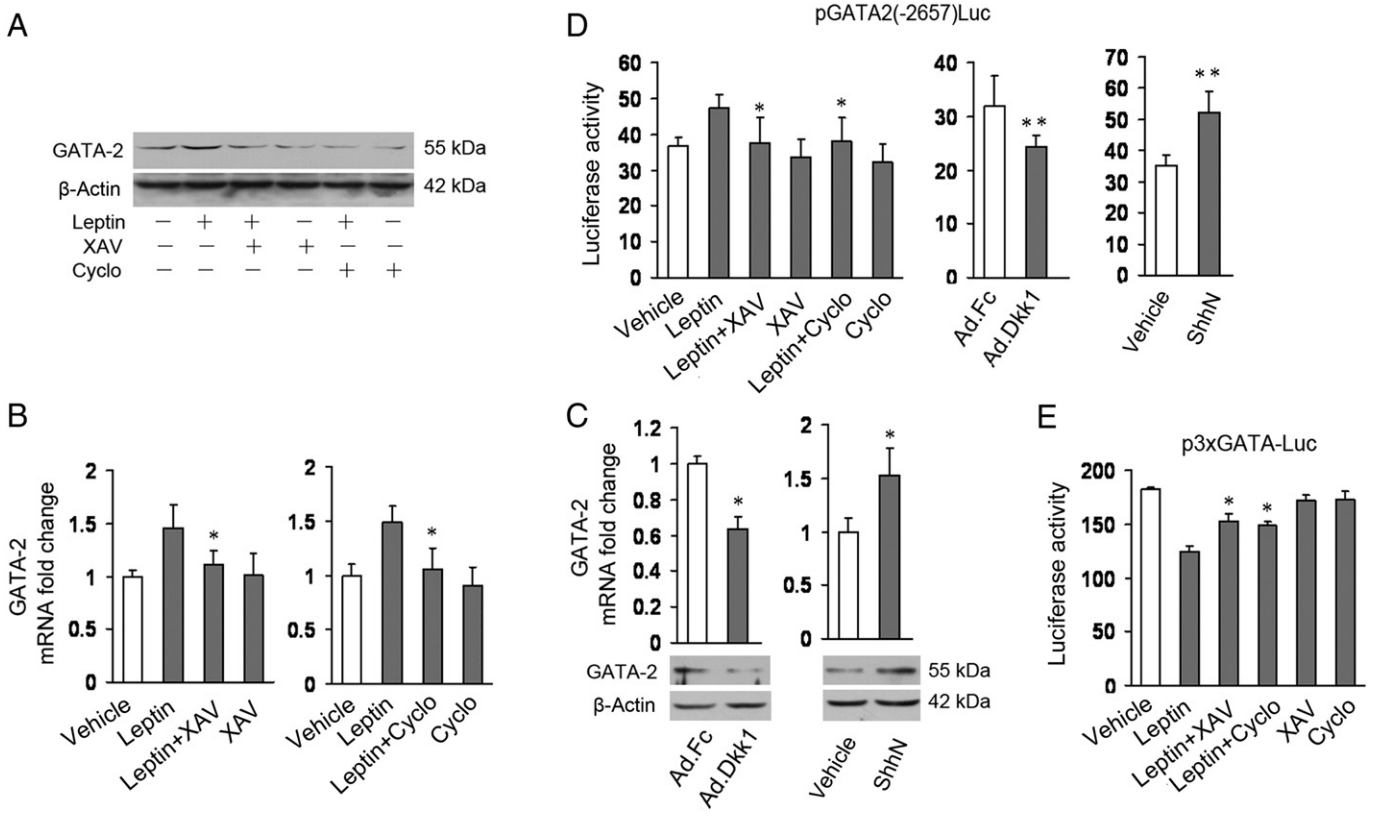

Fig. 4. Both β-catenin pathway and Hh pathway mediate leptin-induced increases in GATA-2 expression and activity. (A, B) Western blot analysis or real-time PCR analysis of GATA-2 expression (n = 3). HSCs were preincubated with or without 5 μM of XAV939 (XAV, inhibiting β-catenin pathway) or cyclopamine (Cyclo, inhibiting Hh pathway) for 1 h before leptin stimulation for 24 h. GATA-2 protein levels and mRNA levels were examined by western blot analysis (A) and real-time PCR analysis (B), respectively. Representative blots were shown and β-actin was used as an internal control. *$P < 0.05$ vs the respective control with leptin alone. (C) Real-time PCR and Western blot analyses of GATA-2 expression (n = 3). HSCs were cultured in six-well plastic plates. The first group of HSCs (the left panel) was transducted with 100 MOI Ad.Dkk1 (for the inhibition of β-catenin pathway) or the control Ad.Fc and incubated with leptin for 24 h. The second group of HSCs (the right panel) was incubated with 100 ng/ml of ShhN or the vehicle for 24 h. The levels of GATA-2 mRNA and protein were examined by real-time PCR and Western blot analyses. *$P < 0.05$ vs the cells with the respective control. (D) Transfection assay for the analysis of GATA-2 promoter activity (n = 3). The first group of HSCs (the left panel) was transfected with 1.6 μg of pGATA2(-2657)Luc and preincubated with or without 5 μM of XAV939 (XAV) or cyclopamine (Cyclo) for 1 h before leptin stimulation for 24 h. The second group of HSCs (the middle panel) was transducted with 1.6 μg of pGATA2(-2657)Luc and 100 MOI Ad.Dkk1 or the control Ad.Fc and then incubated with leptin for 24 h. The third group of HSCs (the right panel) was transfected with 1.6 μg of pGATA2(-2657)Luc and incubated with 100 ng/ml of ShhN or the vehicle for 24 h. Luciferase activities were examined. *$P < 0.05$ vs the cells with leptin alone. **$P < 0.05$ vs the cells with the respective control (Ad.Fc or vector). (E) Transfection assay for analysis of GATA-2 activity (n = 3). HSCs were transfected with 1.6 μg of p3xGATA-Luc and preincubated with or without 5 μM of XAV939 (XAV) or cyclopamine (Cyclo) for 1 h before leptin stimulation for 24 h. Luciferase activities were examined. *$P < 0.05$ vs the cells with leptin alone.

the luciferase activity (right panel in the Fig. 4D). These results in Fig. 4D suggested that the two pathways were also involved in leptin induction of GATA-2 promoter activity.

Furtherly, the effect of leptin-induced β-catenin or Hh pathway on GATA-2 trans-regulatory activity was examined by using p3xGATA-Luc. HSCs were transfected with p3xGATA-Luc and then pretreated with or without XAV939 or cyclopamine before leptin treatment (Fig. 4E). As expected, luciferase assay showed that the inhibition of leptin-induced β-catenin or Hh pathway reduced leptin-induced decrease in luciferase activity (Leptin + XAV or Leptin + Cyclo) as compared with the control with leptin alone, suggesting that the two pathways mediated leptin promotion of GATA-2 trans-regulatory activity.

The results in Fig. 4 indicated that β-catenin pathway and Hh pathway mediated leptin-induced increase in GATA-2 expression and activity in HSCs in vitro.

3.5. Leptin induces β-catenin pathway and Hh pathway, accompanied by the increase in GATA-2 expression and liver fibrosis and the decrease in PPARγ expression in HSCs in TAA-induced liver injury of ob/ob mice

To examine whether leptin treatment influences pathways of β-catenin and Hh and the expressions of GATA-2 and PPARγ in HSCs in vivo (Because PPARγ1 antibody is not available, PPARγ was detected by using PPARγ antibody), we examined the expressions of β-catenin, Shh, GATA-2, and PPARγ in HSCs in TAA-induced liver injury of ob/ob mice. After the mice were given the administration of TAA plus vehicle or TAA plus leptin for 4-week, the β-catenin-, Shh-, GATA-2-, or PPARγ-positive HSCs were detected by double fluorescence staining or HSCs were isolated from the treated mice and directly used for Western blot analysis of β-catenin, Shh, GATA-2, or PPARγ. Fig. 5 showed that the relative number of β-catenin-, Shh-, or GATA-2-positive HSCs in the livers with TAA plus leptin (T + L) significantly increased as compared

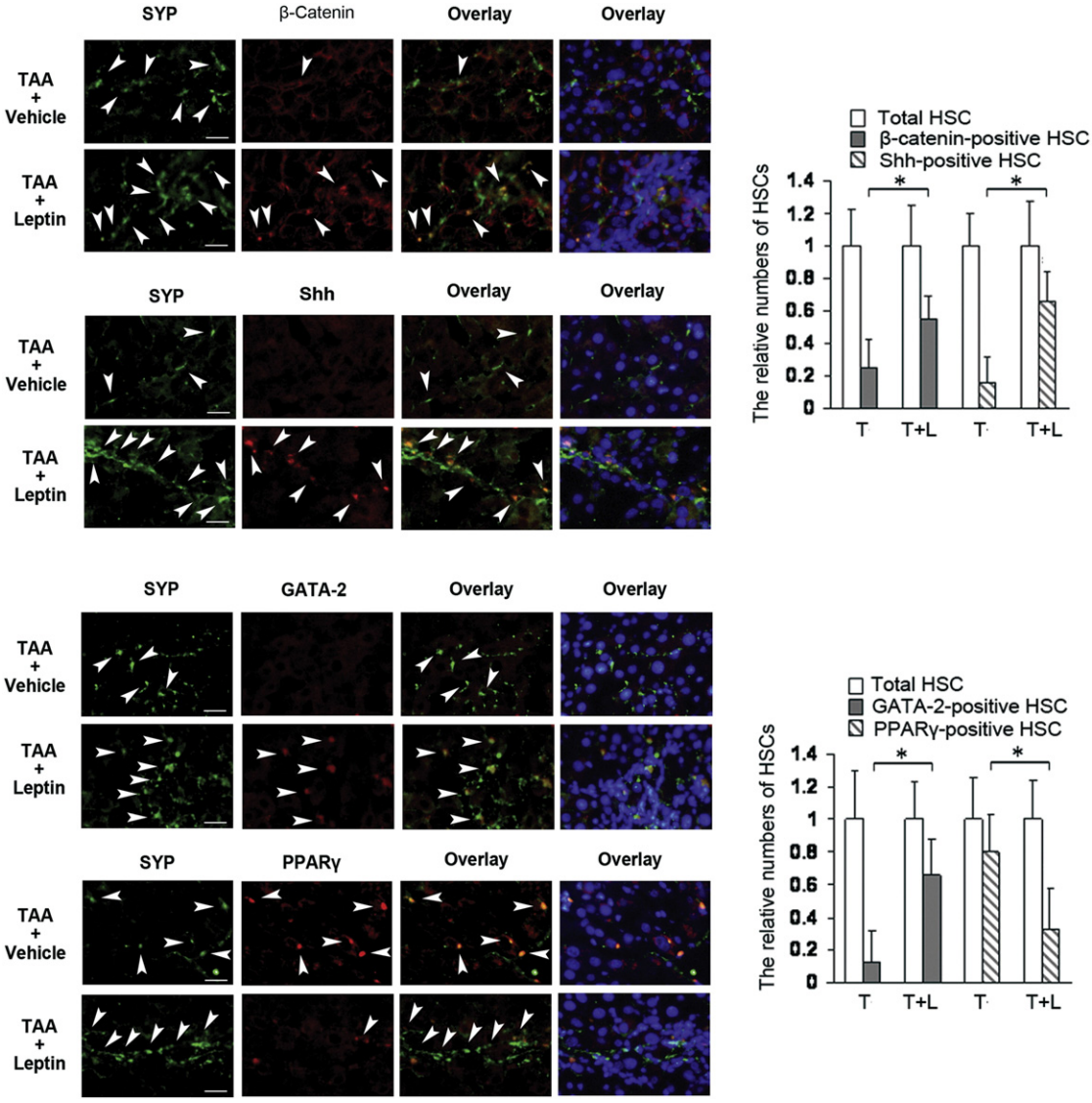

Fig. 5. Leptin stimulates the activation of of β-catenin pathway or Hh pathway, accompanied by the increase in GATA-2 expression and the decrease in PPARγ expression in HSCs in TAA-induced liver injury of ob/ob mice. Two groups of ob/ob mice (6 mice/each group) were given the administration of TAA (200 μg/g body weight, two times a week) plus vehicle or TAA plus leptin (1 μg/g body weight, once per day) by intraperitoneal injection for 4-week. Double fluorescence staining on the liver section was performed for detecting β-catenin-, Shh-, GATA-2-, or PPARγ-positive HSCs by using the respective primary antibody against β-catenin, Shh, GATA-2, or PPARγ plus primary antibody against synaptophysin (SYP, a marker for quiescent and activated HSCs) and subsequently the DyLight594-conjugated secondary antibody (red fluorescence) and DyLight488-conjugated secondary antibody (green fluorescence). The nuclei were counterstained with Hoechst 33342 (blue fluorescence). The representative images were captured with the fluorescence microscope. Scale bar 50 μm. Arrowheads indicated examples of positively stained cells. The total HSCs (SYP-positive HSCs) and β-catenin-, GATA-2-, PPARγ-, or Shh-positive HSCs were counted in six randomly chosen fields at 100-fold magnification and the values were expressed as fold changes relative to the respective total HSCs (empty column) in group treated with TAA plus vehicle (T) or TAA plus leptin (T + L). The values were shown as a histogram on the respective right panel. $^{*}P < 0.05$.

with that in the livers with TAA plus vehicle (T), suggesting that leptin promoted the expressions of β-catenin, Shh, or GATA-2 in HSCs in the model of TAA-induced liver injury. Conversely, the relative number of PPARγ-positive HSCs in the livers with TAA plus leptin significantly decreased as compared with that in the livers with TAA plus vehicle, indicating that leptin reduced PPARγ expression in HSCs in the same model. Western blot analysis by using HSCs isolated from the treated mice demonstrated the same results (Fig. 1 of supplemental data 3) as shown by double fluorescence staining (Fig. 5). We also detected the expressions of inducers of liver fibrosis (TGFβ1, PDGFRβ, MMP2, TIMP1, and TIMP2), inhibitor of liver fibrosis (MMP1), and collagen (shown by hydroxyproline content) in the respective treated livers (the protein levels of α1(I)collagen and α-SMA in the same model have been shown in our previous studies [13]). Western blot analysis showed that leptin enhanced the expressions of TGFβ1, PDGFRβ, MMP2, TIMP1, TIMP2, and hydroxyproline content and reduced MMP1 expression in the livers of mouse model of TAA-induced liver damage (Fig. 1 of supplemental data 3), which supported the results as shown by double fluorescence staining in Fig. 5.

These results suggested that leptin-induced activation in β-catenin pathway or Hh pathway was accompanied by the increase in GATA-2 expression and the decrease in PPARγ expression in HSCs and enhanced liver fibrosis in the mouse model of TAA-induced liver injury. The in vivo results were in line with in vitro results.

3.6. Interruption of leptin-induced β-catenin pathway or Hh pathway leads to the decrease in GATA-2 expression and the increase in PPARγ expression in HSCs in TAA-induced liver injury of ob/ob mice, accompanied by the inhibitions of HSC activation and liver fibrosis

Furtherly, we used the Ad.Dkk1 or cyclopamine to interrupt the respective signaling pathway induced by leptin in the mouse model of TAA-induced liver injury as described in Materials and methods and the expressions of GATA-2 and PPARγ in HSCs (Fig. 6A and B) or the α-SMA-positive cells in livers were detected by immunofluorescence analysis (Fig. 6C) or HSCs were isolated from the treated mice and directly used for Western blot analysis of GATA-2 and PPARγ (Fig. 2 of supplemental data 3). Liver fibrosis was examined by sirius red staining (Fig. 6C). Compared with the respective control (TAA + Leptin + Ad.Fc, T + L, Fig. 6A) or (TAA + Leptin + Vehicle, T + L, Fig. 6B), the relative number of GATA-2-positive HSCs significantly decreased and the relative number of PPARγ-positive HSCs increased in the livers with Ad.Dkk1 (TAA + Leptin + Ad.Dkk1, T + L + Dkk1, Fig. 6A) or cyclopamine (TAA + Leptin + Cyclo, T + L + Cyclo, Fig. 6B). These results suggested that leptin-induced β-catenin pathway or Hh pathway led to the increase in GATA-2 expression and the decrease in PPARγ expression in HSCs in vivo in the model. Western blot analysis by using HSCs isolated from the treated mice demonstrated the same results (Fig. 2 of supplemental data 3) as shown by double fluorescence staining (Fig. 6A and B).

Both the number of α-SMA-positive cells and collagen (shown by sirius red staining) also decreased in the livers with cyclopamine (TAA + Leptin + Cyclo, T + L + Cyclo) as compared with its control without cyclopamine (TAA + Leptin + Vehicle, T + L) (Fig. 6C), which supported the roles of leptin-induced Hh pathway in the expressions of GATA-2 and PPARγ in HSCs in the model. Our previous studies have demonstrated that the interruption of leptin-induced β-catenin pathway by Ad.Dkk1 in the same model reduced α1(I)collagen and the number of activated HSCs [13], therefore, we here have not examined them again. We also detected the expressions of TGFβ1, PDGFRβ, MMP1, MMP2, TIMP1, TIMP2, and collagen (shown by hydroxyproline content) in the treated livers. Western blot analysis showed that the interruption of leptin-induced β-catenin pathway or Hh pathway reduced the expressions of TGFβ1, PDGFRβ, MMP2, TIMP1, TIMP2, and hydroxyproline content and enhanced MMP1 expression in the livers of mouse model of TAA-induced liver damage (Fig. 2 of supplemental data 3), which supported the results as shown by double fluorescence staining in Fig. 6.

4. Discussion

Leptin plays a unique role in promoting liver fibrosis in vitro and in vivo [9–13] and elicits an inhibitory effect on the expression of PPARγ, a key TF for inhibiting HSC activation, in HSCs [14,15]. The present studies demonstrated the mechanisms by which leptin down-regulated PPARγ1 in HSCs. Results suggested that: 1) Leptin stimulated the expression of GATA-2 in vivo and in vitro; 2) GATA-2 could bind to the PPARγ1 promoter at a site around position −2323 in PPARγ1 promoter and inhibit PPARγ1 promoter activity in vitro; 3) GATA-2 mediated leptin inhibition of PPARγ1 expression by GATA-2 binding site (around position −2323) of PPARγ1 promoter and GATA-2 promoted HSC activation in vitro; and 4) leptin up-regulated GATA-2 expression, at least in part, through β-catenin pathway and Hh pathway in vivo and in vitro. 5) leptin-induced increase in GATA-2 was accompanied by the decrease in PPARγ expression in HSCs and by increase in the activated HSC number and liver fibrosis in ob/ob mouse model.

PPARγ includes two subtypes, namely PPARγ1 and PPARγ2. The two subtypes arise from the use of different promoters and alternative splicing [27]. PPARγ1 is the major form of PPARγ in HSCs [16]. Our researches showed that GATA-2 inhibited PPARγ1 expression in HSCs and identified a site around position −2323 of PPARγ1 promoter as GATA-2 binding site. Moreover, the binding site for GATA-2 was one of leptin response elements in PPARγ1 promoter. These results were based on multi-level experiments: 1) the progressive 5′-deletion, point mutation, gain- and loss-of-function of GATA-2, EMSA, and ChIP; 2) leptin-induced increase in GATA-2 expression in vitro and in vivo.

TFs of GATA binding proteins have been found to serve as negative regulators of adipocyte formation [24,25]. GATA-2, a member of GATA binding protein family, suppresses adipocyte differentiation [25,26]. More importantly, it traps cells at the preadipocyte stage and the change of GATA-2 expression is the early events in the commitment of the preadipocyte to differentiate [26], suggesting that GATA-2 functions as a crucial TF in controlling adipocyte differentiation. PPARγ is considered as the central engine of adipocyte differentiation [28]. Quiescent HSCs are much like adipocyte and HSC activation is analogous to adipocyte to preadipocyte transdifferentiation [2]. We here showed the relationship between GATA-2 and PPARγ1 in HSCs.

CCAAT/enhancer binding α (C/EBPα) is another important TF in promotion of adipocyte differentiation [23] and can also reduce HSC activation and liver fibrosis [29]. GATA-2 is found to bind to C/EBPα protein and thus inhibits C/EBPα trans-activation activity in NIH 3T3 cells [30], which is different from the mechanisms underlying GATA-2 inhibition of PPARγ1.

Since HSC activation is analogous to adipocyte to preadipocyte transdifferentiation and GATA-2 can trap cells at the preadipocyte stage [26], there is a possibility that GATA-2 might play an important role in controlling HSC activation. Our results demonstrated an inhibitory effect of GATA-2 on the expression of PPARγ1, a major form of PPARγ in HSCs and a key TF in inhibiting HSC activation, and showed that GATA-2 knockdown led to the decreases in the expressions of α-SMA (a well-established marker for HSC activation) and α1(I) collagen (the major component of ECM) in HSCs in vitro. Furthermore, leptin-induced increase in GATA-2 was accompanied by the decrease in PPARγ expression in HSCs and by increase in the activated HSC number and liver fibrosis in vivo. These results implied the promotion role of GATA-2 in leptin-induced mouse liver fibrogenesis.

GATA-3, a member of GATA binding proteins, appears to enhance the development of pulmonary fibrosis [31]. GATA-6 also mediates the profibrotic, myofibroblast-like differentiation signal of transforming growth factor-β1 (TGF-β1) in lung fibroblast [32]. We reported here that GATA-2 suppressed the expression of PPARγ1, a key TF for inhibiting HSC activation, and contributed to HSC activation and

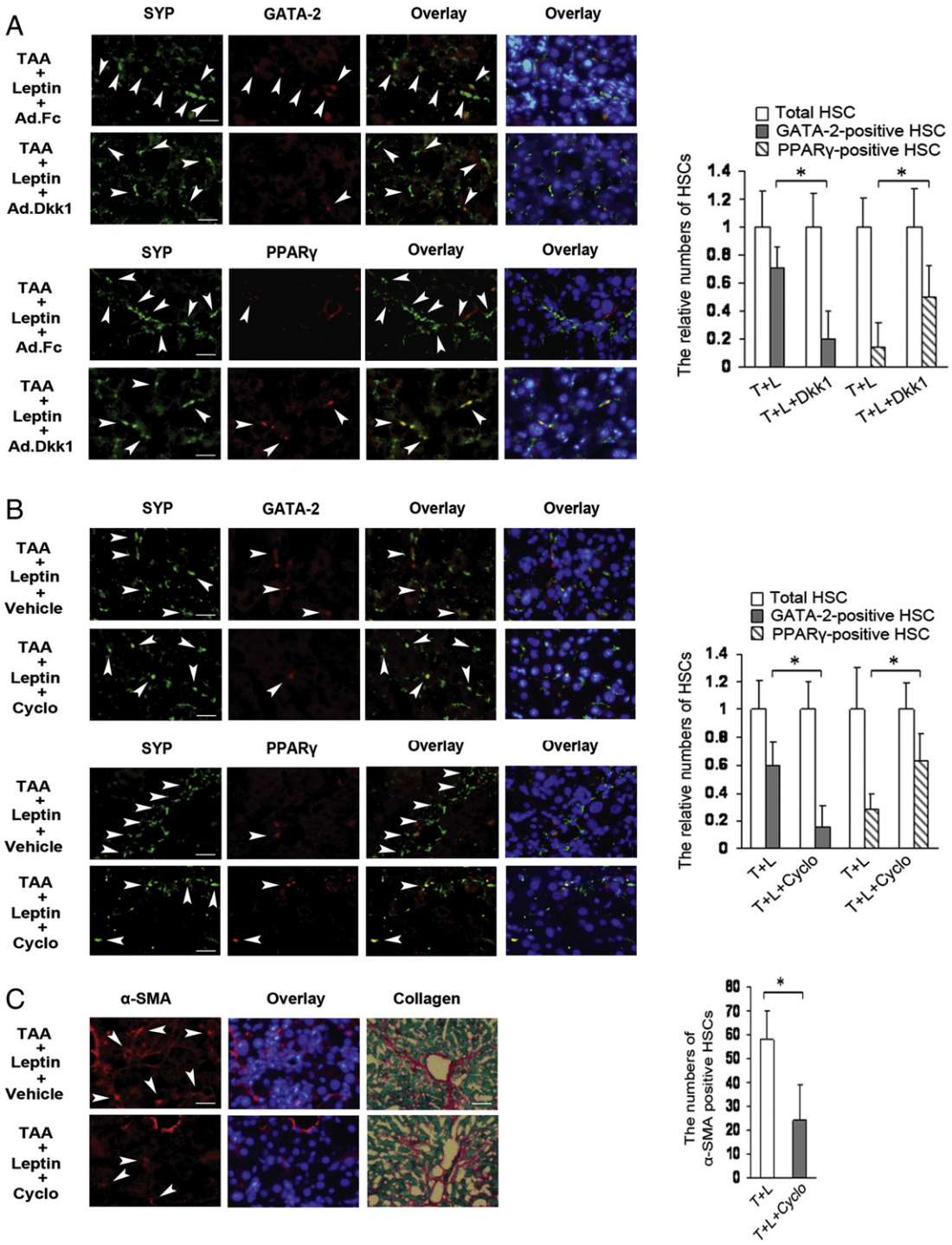

Fig. 6. Interruption of leptin-induced the pathway of β-catenin or Hh leads to the decrease in GATA-2 expression and the increase in PPARγ expression in HSCs, accompanied by the inhibitions of HSC activation and liver fibrosis in TAA-induced liver injury of ob/ob mice. The first two groups (ob/ob mice, 6 mice/each group) were treated with Ad.Dkk1 (inhibiting β-catenin pathway, 2×10^7 pfu/g body weight, once every two weeks) or Ad.Fc (a control adenovirus) throughout the 4-week period of TAA plus leptin treatment (A) as described in Fig. 5. The second two groups were treated with cyclopamine (Cyclo, inhibiting Hh pathway, 1 μg/g body weight, once per day) or vehicle throughout the 4-week period of TAA plus leptin treatment (B). Double fluorescence staining on the liver section was performed for detecting GATA-2- or PPARγ-positive HSCs as described in Fig. 5. Single fluorescence staining on the liver sections was used to detect α-SMA by the primary antibody against α-SMA and the DyLight594-conjugated secondary antibody (red fluorescence) or liver fibrosis was examined by sirius red staining of collagen (C). The nuclei were counterstained with Hoechst 33342 (blue fluorescence). The representative images were captured with the fluorescence microscope or a light microscope. Scale bar 50 μm. Arrowheads indicated examples of positively stained cells. The total HSCs (SYP-positive HSCs) and GATA-2- or PPARγ-positive HSCs were counted in six randomly chosen fields at 100-fold magnification and the values were expressed as fold changes relative to the respective total HSCs (empty column) in group treated with TAA + Leptin + Ad.Fc (T + L) (A), TAA + Leptin + Ad.Dkk1 (T + L + Dkk1) (A), TAA + Leptin + Vehicle (T + L) (B), or TAA + Leptin + Cyclo (T + L + Cyclo) (B). The values were shown as a histogram on the respective right panel (A and B). The α-SMA-positive HSCs were counted in six randomly chosen fields at 100-fold magnification and the average values were shown as a histogram on the right panel (C). $^*P < 0.05$.

collagen expression. These data suggested that some of the GATA binding proteins might be not only involved in adipocyte differentiation, but are also correlated with organ fibrogenesis.

Leptin is an adipocyte-derived hormone and links nutrition, metabolism, and immune homeostasis [33,34]. It can suppress lipid synthesis in preadipocyte [35] and reverse adipocyte differentiation [36]. In this

report, leptin was shown to induce GATA-2 expression in HSCs. Considering the key role of GATA-2 in controlling adipocyte differentiation, the influence of leptin on GATA-2 expression in HSCs seems to be in line with its inhibitory effect on lipid synthesis and adipocyte differentiation. In addition to the role of leptin in GATA-2 expression in HSCs, leptin also induces GATA-4 activation in cardiomyocytes [37]. TGF-β is a key cytokine in the promotion of HSC activation and its signaling pathway can be mediated by Smad3 [38]. GATA-4, interacting with Smad3, is a cofactor for TGF-β signaling in granulosa cells [39]. Therefore, it is worth testing whether leptin also affects HSCs by GATA-4.

Interestingly, both β-catenin signaling and Hh signaling are demonstrated to inhibit adipocyte differentiation [40–42] and are also involved in the promotion of liver fibrosis [11,43,44]. The activation of β-catenin signaling pathway is required for liver fibrosis [13], pulmonary fibrosis [45], and skin fibrosis [46]. The severity of liver fibrosis parallels the level of Hh pathway activity in patients with different types of liver disease [47]. Hh signaling pathway is suggested as a regulator of adult liver repair [48]. It should be noted that the two pathways correlate with the promotion effects of leptin on HSC activation [11,13]. Our results showed that leptin-induced activation of β-catenin pathway or Hh pathway promoted GATA-2 expression in HSCs in vitro and in vivo and GATA-2 could bind to PPARγ1 promoter, thus inhibiting the expression of PPARγ1, a key TF in the inhibition of HSC activation. Data from this study might suggest a mechanism underlying the role of the two pathways in PPARγ expression in HSCs and in rodent liver fibrogenesis.

5. Conclusions

In summary, this report demonstrated that leptin stimulation induced GATA-2 expression by β-catenin signaling pathway and Hh signaling pathway in HSCs and consequently GATA-2 bound to PPARγ1 promoter at site around position −2323, thus inhibiting PPARγ1 expression. GATA-2 might be correlated with the effects of leptin on HSC activation and on the mouse liver fibrosis. These results do not exclude the other mechanisms underlying leptin regulation of PPARγ1 expression in HSCs. Our data suggest a possible new explanation for the promotion effect of leptin on liver fibrogenesis.

Conflict of interest

None declared.

Acknowledgments

The authors thank Dr. Jill A. Helms (Stanford University) for the gifts of Ad.Fc and Ad.Dkk1.

This work was supported by the Natural Science Foundation of China (81270512); and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institution (PAPD).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbadis.2014.10.001.

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