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Hepatocyte Peroxisome Proliferator—Activated Receptor α Enhances Liver Regeneration after Partial Hepatectomy in Mice

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Peroxisome proliferator—activated receptor α (PPARα) is a key nuclear receptor involved in the control of lipid homeostasis. In rodents, PPARα is also a potent hepatic mitogen. Hepatocyte-specific disruption of PPARα inhibits agonist-induced hepatocyte proliferation; however, little is known about the exact role of PPARα in partial hepatectomy (PHx)—induced liver regeneration. Herein, using hepatocyte-specific PPARα-deficient (*Ppara*$^{\Delta Hep}$) mice, the function of hepatocyte PPARα in PHx-induced liver regeneration was investigated. PPARα protein level and transcriptional activity were increased in the liver after PHx. Compared with the *Ppara*$^{fl/fl}$ mice, *Ppara*$^{\Delta Hep}$ mice exhibited significantly reduced hepatocyte proliferation at 32 hours after PHx. Consistently, reduced *Ccnd1* and *Pcna* mRNA and CYCD1 and proliferating cell nuclear antigen protein were observed at 32 hours after PHx in *Ppara*$^{\Delta Hep}$ mice. Furthermore, *Ppara*$^{\Delta Hep}$ mice showed increased hepatic lipid accumulation and enhanced hepatic triglyceride contents because of impaired hepatic fatty acid β-oxidation when compared with that observed in *Ppara*$^{fl/fl}$ mice. These results indicate that PPARα promotes liver regeneration after PHx, at least partially via regulating the cell cycle and lipid metabolism. *(Am J Pathol 2019, 189: 272—282; https://doi.org/10.1016/j.ajpath.2018.10.009)*

The liver has a remarkable ability to regenerate. A 70% partial hepatectomy (PHx) animal model is commonly used to study liver regeneration. After PHx, the hepatocyte, the main cell type of the liver, replicates through both hypertrophic and hyperplastic mechanisms.[1] Liver regeneration is a compensatory hyperplasia process involving multiple growth factors, cytokines, hormones, and nuclear receptors.[2–8] Almost all the cell types in liver participate in the PHx response; hepatocytes and cholangiocytes rapidly enter into the cell cycle, whereas stellate cells, Kupffer cells, and endothelial cells follow hepatocytes in the procession of regeneration,[8] which takes 5 to 7 days in mice, with cell proliferation peaking at days 2 or 3.[2–4,8] In the early stage after liver regeneration, the blood concentrations of hepatocyte growth factor, epidermal growth factor, tumor necrosis factor α (TNFα), IL-6, norepinephrine, bile acids, and serotonin were rapidly increased.[2–7] It is widely accepted that PHx causes a transient increase in lipopolysaccharide levels, which stimulates Kupffer cells to produce IL-6 and TNFα that act on hepatocytes to induce survival and proliferation.[9,10]

Systemic and liver local metabolic changes are believed to trigger and participate in liver regeneration. Hypoglycemia

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G.X. and S.Y. contributed equally to this work.

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Table 1 List of Real-Time Quantitative PCR Primer Sequences

Genes	NCBI accession number*	Forward	Reverse
Ppara	NM_001113418	5′-CTGCCTTCCCTGTGAACTGA-3′	5′-ACAGAGCGCTAAGCTGTGAT-3′
Ccna2	NM_009828	5′-TCGCTGCATCAGGAAGACC-3′	5′-CTTAAGAGGAGCAACCCGTCG-3′
Ccnd1	NM_007631	5′-TCAAGTGTGACCCGGACTGC-3′	5′-CCTTGGGGTCGACGTTCTG-3′
Ccne1	NM_007633	5′-ACTTCCCGTCTTGAATTGGGG-3′	5′-AGGATGACGCTGCAGAAAGT-3′
Pcna	NM_011045	5′-TCGTCTCACGTCTCCTTGGT-3′	5′-TTTTGGACATGCTGGTGAGGT-3′
Check1	NM_007691	5′-CTGGCAAAGGACTGCTTGTC-3′	5′-GTTGAACTTCTCCATAGGCACC-3′
Mcm2	NM_008564	5′-CAGCATTGCACCCTCCATCT-3′	5′-TGCTTTCCACCTGGGTTCTT-3′
Mcm5	NM_001302540	5′-AACGAACCAATAGGAGCGCA-3′	5′-ATGACTGTACCTCAGCCCTC-3′
Prkdc	NM_011159	5′-ACAGAGACGGTAATCACGGGT-3′	5′-CCAGCAGGAAAGCTGGGTT-3′
Rad51	NM_011234	5′-TTCACGGTTAGAGCAGTGTGG-3′	5′-TTCGGTGCATAAGCAACAGC-3′
G6pc	NM_008061.4	5′-GTCTTGTCAGGCATTGCTGTG-3′	5′-GAATCCAAGCGCGAAACCAA-3′
Pck1	NM_011044.2	5′-ATGAAAGGCCGCACCATGTA-3′	5′-GGGCGAGTCTGTCAGTTCAA-3′
Pkm	NM_001253883.1	5′-ATGCAGCACCTGATAGCTCG-3′	5′-AGGTCTGTGGAGTGACTGGA-3′
Pfkm	NM_001163487.1	5′-GGAGAGCTAAAACTACAAGAGTGG-3′	5′-CTCCACCAGAGGTCAACACG-3′
Gck	NM_001287386.1	5′-CCTCGGGAGTCAGGAACATC-3′	5′-ATCTGCTCTACCAGAGTCAACG-3′
Hadha	NM_178878.2	5′-AAGAGCTTTCGTCCTCTTCTGC-3′	5′-AAATGCAGCCTCTGGAGCGTA-3′
Hadhb	NM_001289798.1	5′-CGGACGTTTGTCAGTCTGGA-3′	5′-CTGAAATCTGCCTGTGGGGA-3′
Cyp4a10	NM_010011.3	5′-AGGAGCCAGGAACTGCATTG-3′	5′-GACCCTGGTAGGATCTGGCA-3′
Cyp4a14	NM_007822.2	5′-TTGCTCACGAGCACACAGAT-3′	5′-TCCTCCATTCTGGCAAACAAGA-3′
Cpt2	NM_009949.2	5′-ATCTCAGGCCCCTGGTTTGA-3′	5′-ATCTCAGGCCCCTGGTTTGA-3′
Ehhadh	NM_023737.3	5′-CGGTCAATGCCATCAGTCCA-3′	5′-AGCACCTGCACAGAAGTTGT-3′
Hmgcs2	NM_008256.4	5′-AGAAATCCCTGGCTCGGTTG-3′	5′-AGCTTTAGACCCCTGAAGGC-3′
Txnip	NM_001009935.2	5′-GAAGGCTTTTCTCGATCGCC-3′	5′-GGCAGACACTGGTGCCATTA-3′
Vnn1	NM_011704.3	5′-GCATGCTGTGATCCTGCCTAA-3′	5′-TAATGTGCGCACCCTGCT-3′
Cpt1a	NM_013495.2	5′-TCGGTGAGCCTGGCCT-3′	5′-TTGAGTGGTGACCGAGTCTG-3′

*Available from *https://www.ncbi.nlm.nih.gov/nuccore*.
NCBI, National Center for Biotechnology Information.

and increased circulating insulin levels also occur soon after PHx.[11–13] The liver presents transient steatosis on day 2 or 3 after PHx, which could influence liver regeneration.[11,14] An early study shows that mice rapidly develop hypoglycemia after the operation, which causes adipose tissue lipolysis and leads to periphery lipid redistribution in the regenerating liver.[15] This study suggests that hepatic steatosis after PHx could promote liver regeneration.[15] However, liver regeneration was impaired after PHx in hepatic steatosis mice,[16] mice with genetic obesity,[17] or mice fed with a high-fat diet.[18] Disruption of hepatic lipid accumulation also decreases liver regeneration after PHx in mice.[19] Until now, the underlying mechanisms of these results remain unclear.

Many regulators of lipid droplet formation and peripheral lipid mobilization were reported to participate in liver regeneration, such as farnesoid X receptor,[20,21] caveolin-1,[22,23] and peroxisome proliferator–activated receptors (PPARs).[24–26] PPARα is a ligand-activated nuclear receptor, which plays an important role in regulating liver and skeletal muscle fatty acid metabolism and glucose homeostasis.[27] Short exposure of mice and rats to peroxisome proliferator chemicals and fibrate drugs leads to hepatocyte proliferation and hepatomegaly through activation of PPARα.[28–30] Chronic exposure to hypolipidemia drugs, such as WY-14643 and fenofibrate, results in prolonged PPARα activation, leading to hepatocellular carcinoma in mice and rats.[31] A previous study revealed that increased PPARα signaling influenced the cell cycle regulation of late-phase liver regeneration.[32] However, using PPARα whole-body knockout mice (*Ppara*-null mice), one report found a delayed hepatocyte proliferation after PHx, accompanied by decreased expression of *Ccnd1* and *Myc* involved in cell cycle regulation and impaired RAS membrane association, which indicate that PPARα could promote cell proliferation after PHx.[26,31] In summary, the findings from these studies suggest that PPARα plays a key role in the liver regeneration program, but the definitive role of PPARα in liver cell proliferation after PHx remains unresolved. A recent study revealed that WY-14643–activated PPARα induces cell proliferation, which is mainly dependent on hepatocyte PPARα,[33] rather than PPARα expressed in nonparenchymal cells, indicating the function of PPARα in hepatocyte proliferation is cell type specific. However, the precise effect of hepatocyte PPARα on liver regeneration after PHx remains unknown. In this study, using mice with conditional ablation of PPARα in hepatocyte ($Ppara^{\Delta Hep}$ mice), hepatocyte PPARα was found to promote liver regeneration after PHx via regulating cell cycle and lipid metabolism.

Materials and Methods

Animals

Male 8- to 10-week–old mice were used in the present study. Hepatocyte-specific PPARα-deficient mice ($Ppara^{\Delta Hep}$

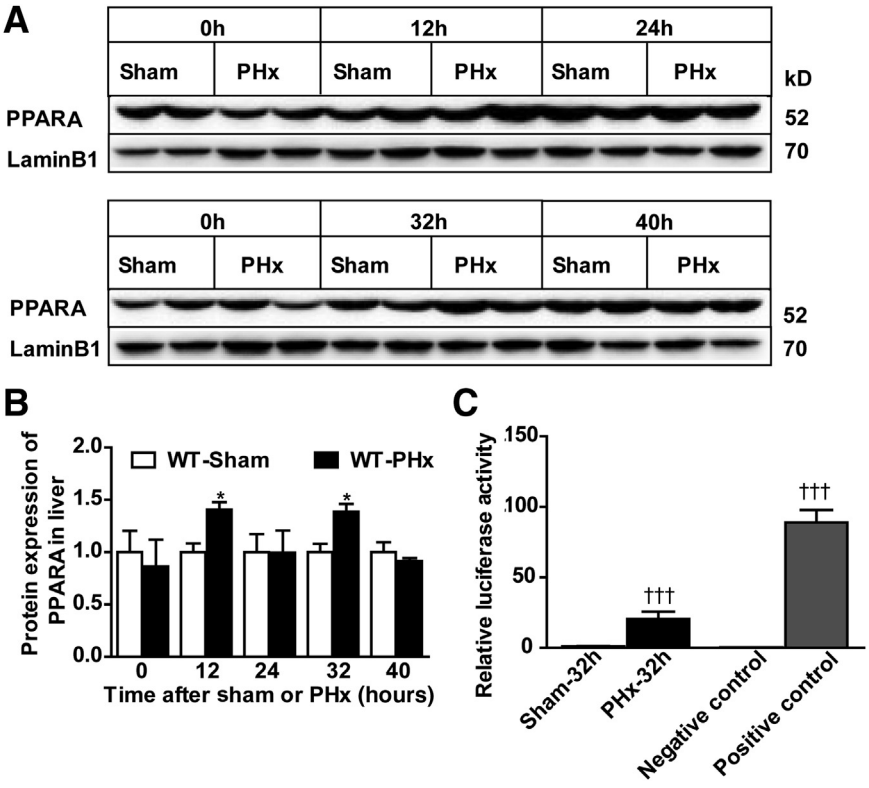

Figure 1 Increased PPARα activity in wild-type (WT) mouse liver after PHx. **A** and **B:** Western blot analysis of PPARα protein expression in WT livers after PHx or sham operation. **C:** Luciferase activity of PPARA response element repeat mice after sham or PHx, negative control, and positive control. Data are expressed as means ± SD (**B** and **C**). $n = 3$ (**A** and **B**); $n = 5$ (**C**). $^{*}P < 0.05$ versus WT-sham; $^{\dagger\dagger\dagger}P < 0.001$ versus sham or negative control.

mice) were generated, as described,[33] and *Ppara*$^{fl/fl}$ mice were used as littermate controls. These mice were kept on a standard 12-hour light/dark cycle with free access to chow diet and water. The animal studies were performed in accordance with protocols approved by the Capital Medical University Animal Care and Use Committee.

To investigate the transcriptional activity of PPARα after PHx, transgenic mice containing a transgene expressing luciferase under control of a PPARA response element repeat were used. These mice were generated, as previously described,[34] and obtained from Charles River Company (Boston, MA). The animal studies were performed in the Laboratory of Metabolism, National Cancer Institute, NIH (Bethesda, MD), in accordance with protocol approved by the National Cancer Institute Animal Care and Use Committee.

Partial Hepatectomy Model and Tissue Harvesting

The two-third partial hepatectomy (PHx) surgery was performed as previously described.[35–37] Mice were injected with 5-bromo-2-deoxyuridine (50 mg/kg body weight) 2 hours before sacrificing at the indicated time points. Liver tissues were formalin fixed or frozen in OCT for cryosection, whereas the remaining liver tissue was snap frozen for further analysis.

Hematoxylin-Eosin Staining and Immunohistochemistry Staining

Paraffin-embedded liver tissues were cut into sections (4 μm thick) for hematoxylin-eosin and immunohistochemistry staining. Hematoxylin-eosin staining was performed following standard methods. Immunohistochemistry analysis was performed using antibodies against 5-bromo-2-deoxyuridine (BD Bioscience, San Jose, CA), as previously described.[9]

Oil Red O Staining

For oil red O staining, OCT-embedded blocks were cut into sections (10 μm thick) and stained with oil red O solution, as previously described.[38]

Quantitative Real-Time RT-PCR

Total RNA was extracted from *Ppara*$^{fl/fl}$ and *Ppara*$^{\Delta Hep}$ livers using Trizol reagent (Life Technologies, Carlsbad, CA), then reverse transcripted into cDNA with GoScript Reverse Transcriptase (Promega, Madison, WI) and used for quantitative real-time RT-PCR analysis with SYBR Green premix (TaKaRa, Nojihigashi, Kusatsu, Shiga, Japan). Quantitative real-time RT-PCR assays were performed on CFX Connect Real-Time System (Bio-Rad, Hercules, CA). Expression of

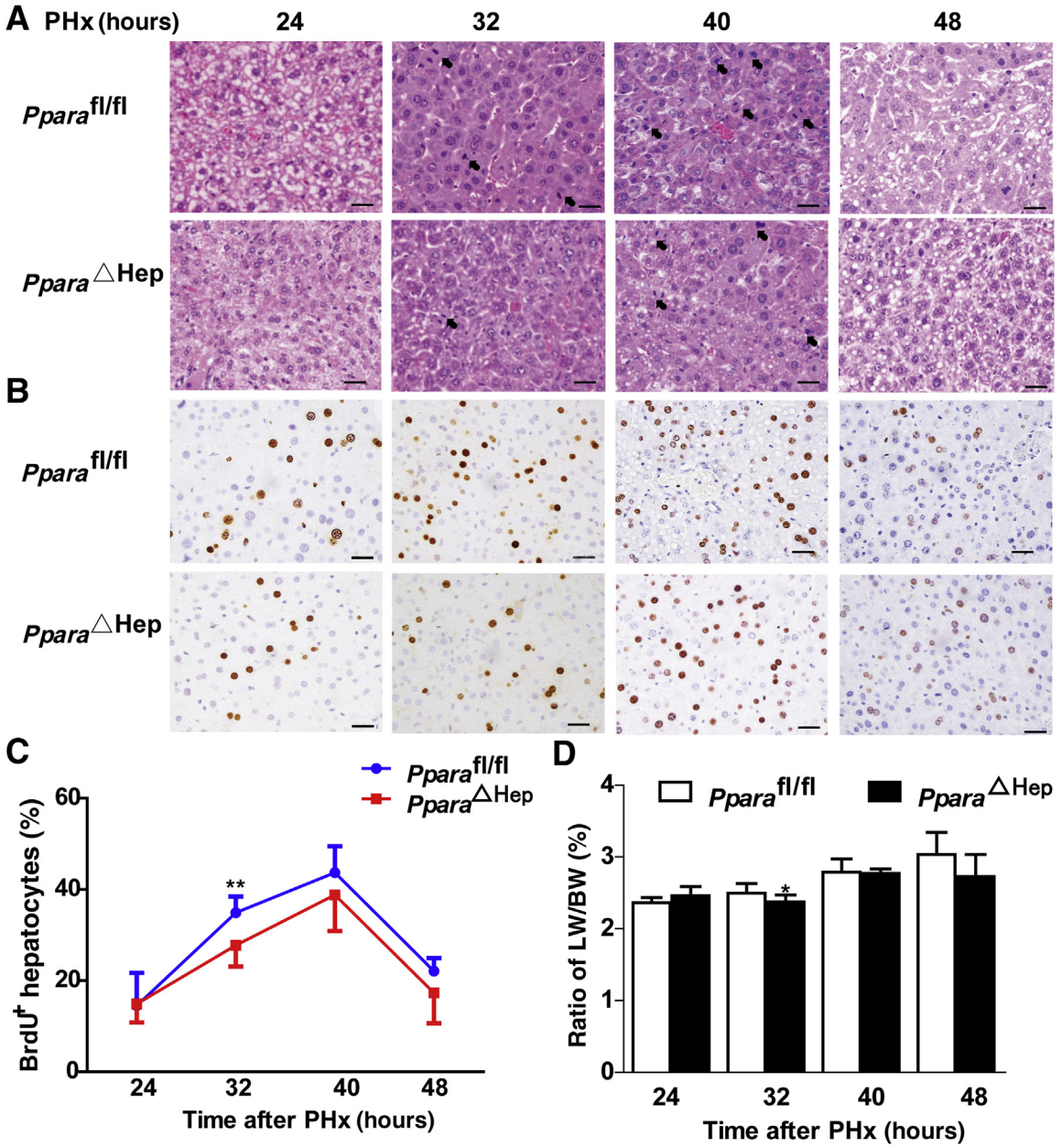

Figure 2 Disruption of PPARα in hepatocyte inhibits liver regeneration after PHx. **A:** Representative images of hematoxylin and eosin staining of liver tissues from $Ppara^{\Delta Hep}$ mice and $Ppara^{fl/fl}$ mice at 24, 32, 40, and 48 hours. The **arrows** refer to mitotic hepatocytes. **B:** Representative images of 5-bromo-2-deoxyuridine (BrdU) staining of residual liver in two groups of mice at 24, 32, 40, and 48 hours after PHx. **C:** BrdU-positive hepatocyte/total hepatocyte ratios per field of two groups at indicated time points. **D:** The ratio of liver weight/body weight (LW/BW) of the two groups at different time points. Data are expressed as means ± SD (**D**). $n = 5$ $Ppara^{fl/fl}$ mice; $n = 11$ $Ppara^{\Delta Hep}$ mice at 24, 32, 40 and 48 hours after PHx. $^{*}P < 0.05$, $^{**}P < 0.01$ versus $Ppara^{fl/fl}$ mice. Scale bars = 20 μm (**A** and **B**). Original magnification, ×400 (**A** and **B**).

target genes was normalized to that of the housekeeping gene β-actin mRNA. The primers are listed in Table 1.

Protein Extraction and Western Blot Analysis

Whole-cell lysate was extracted using radioimmunoprecipitation assay buffer (Applygen, Beijing, China), and nuclear extraction was done with a nuclear and cytoplasmic protein extraction kit (KeyGENBioTECH, Nanjing, China). The protein concentration was measured using the bicinchoninic acid protein assay kit (Thermo Scientific, Waltham, MA). Specific primary antibodies used were as follows: antibody against PPARα and LaminB1 from Abcam (Cambridge, UK), cyclin D1 and β-actin antibodies from Cell Signaling Technology (Boston, MA), and proliferating cell nuclear antigen (PCNA) antibody from Santa Cruz Biotechnology (Dallas, TX). The dilutions were 1:1000 in 5% bovine serum albumin. After incubating

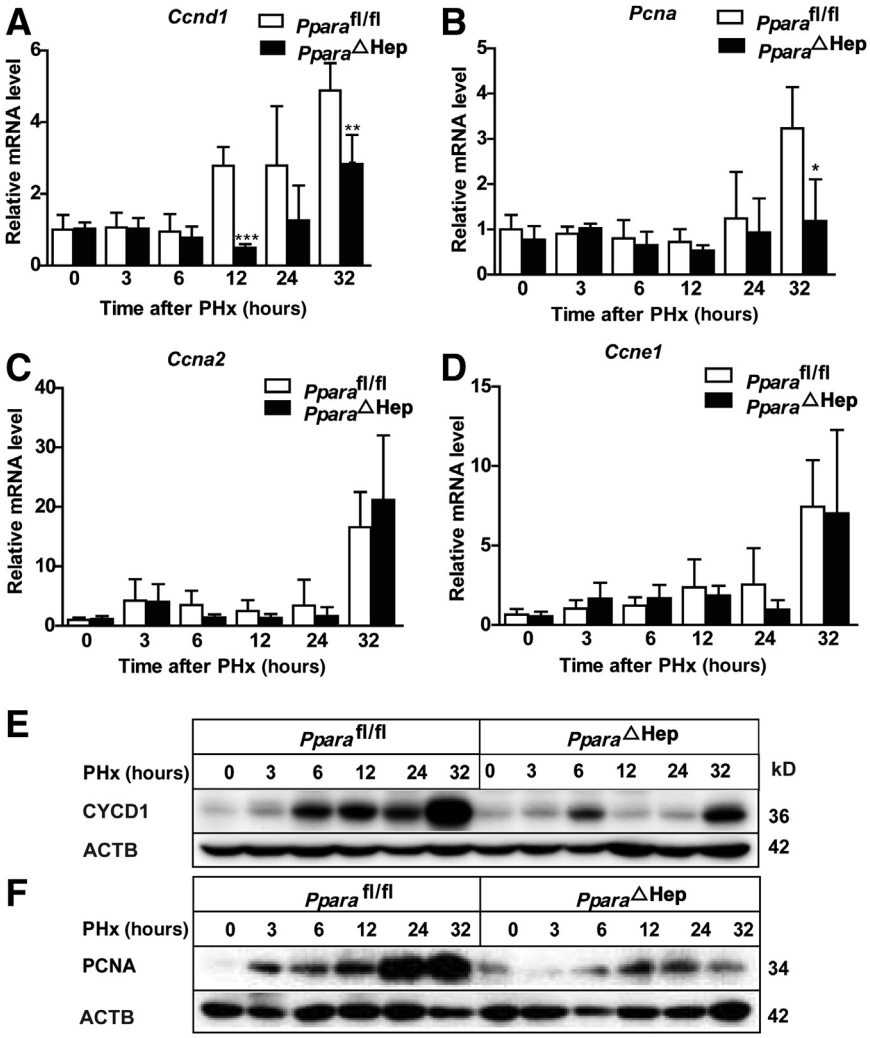

Figure 3 Disruption of PPARα in hepatocytes results in decreased cell cycle gene expression after PHx. **A**—**D:** Real-time PCR analysis of cell cycle—related genes *Ccna2*, *Ccnd1*, *Ccne1*, and *Pcna* mRNA levels in $Ppara^{\Delta Hep}$ and $Ppara^{fl/fl}$ mice at different time points after PHx. **E** and **F:** Western blot analysis of CYCD1 and proliferating cell nuclear antigen (PCNA) protein expression in $Ppara^{\Delta Hep}$ mice and $Ppara^{fl/fl}$ mice at 0, 3, 6, 12, 24, and 32 hours after PHx. Data are expressed as means ± SD (**A**—**D**). $n = 5$ (**A**—**D**). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus $Ppara^{fl/fl}$. ACTB, β-actin.

with horseradish peroxidase—conjugated secondary antibody, the immunocomplexes were visualized with FluorChem-R (ProteinSimple, San Jose, CA). Total protein levels were normalized to β-actin (ACTB), and nuclear protein levels were normalized to LaminB1.

Microarray Analysis

Microarray analysis was performed by CNKINGBIO Company (China). Total RNA was extracted and purified, and the quality was examined. Purified RNA was labeled and hybridized to an AffymetrixHuman Gene 1.1 ST array plate (Affymetrix, Santa Clara, CA). Hybridization, washing, and scanning were performed on an Affymetrix GeneTitan platform following the instruction. Kyoto Encyclopedia of Genes and Genomes is a knowledge base for systematic analysis of gene functions, linking genomic information with higher-order functional information, and now is used widely for pathway-related analysis. The hypergeometric distribution was used to calculate the pathway enrichment, and false discover rate was used to adjust the *P* values for multiple comparisons.

Hepatic Triglyceride Measurement

Hepatic triglycerides were extracted in 50 mmol/L Tris buffer, homogenized, and incubated at 37°C with shaking overnight. A triglyceride measurement kit (Applygen) was used following the manufacturer's instruction to measure triglyceride contents.

Serum β-Hydroxybutyrate Assay

Serum concentrations of β-hydroxybutyrate were detected by enzyme-linked immunosorbent assay (NanJingJianCheng Bioengineering Institute, Nanjing, China), strictly according to the manufacturer's instruction.

Luciferase Assay

PPARA response element repeat mice, aged 8 to 10 weeks, were subjected to PHx or sham surgery, as outlined in the original protocol. Thirty-two hours after surgery, livers were harvested and luciferase activity was measured by the Dual-Luciferase Assay System and normalized by protein

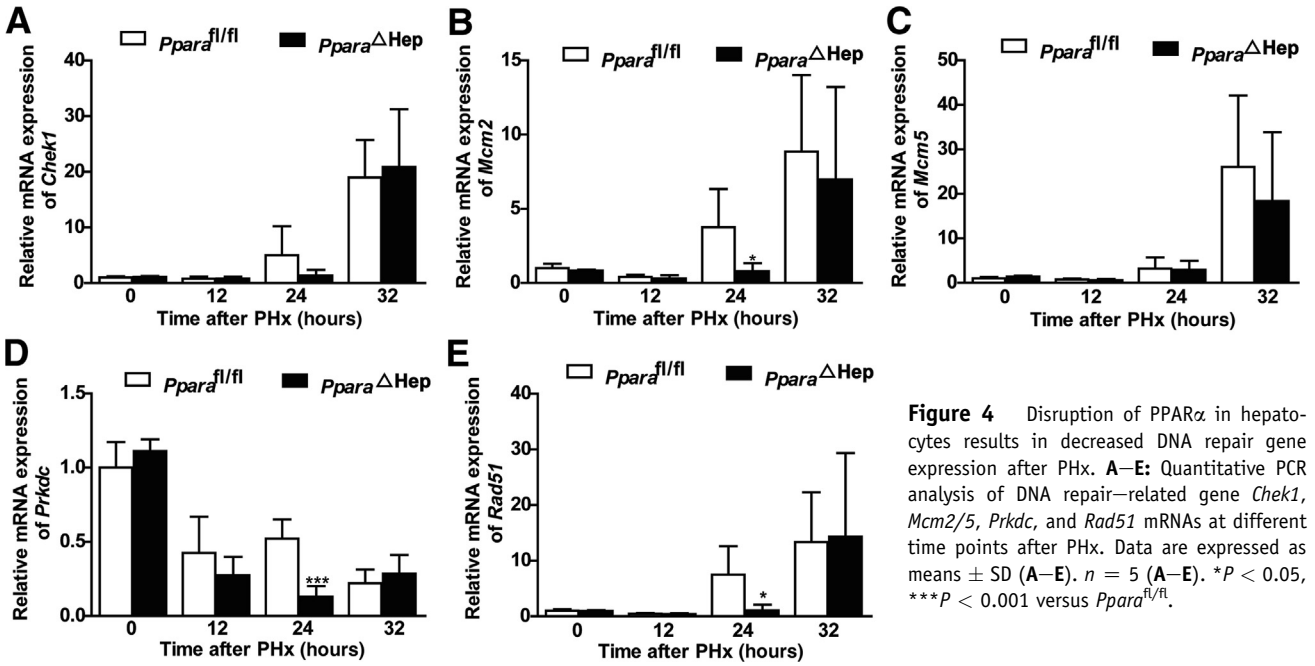

Figure 4 Disruption of PPARα in hepatocytes results in decreased DNA repair gene expression after PHx. **A**—**E:** Quantitative PCR analysis of DNA repair—related gene *Chek1*, *Mcm2/5*, *Prkdc*, and *Rad51* mRNAs at different time points after PHx. Data are expressed as means ± SD (**A**—**E**). $n = 5$ (**A**—**E**). $^{*}P < 0.05$, $^{***}P < 0.001$ versus $Ppara^{fl/fl}$.

concentration. PPARA response element repeat mice treated with PPARα agonist Wy-14643 were served as positive control, and nontreatment served as negative control.

Statistical Analysis

All data are presented as means ± SD. Significant difference between groups was determined using unpaired *t*-test. Values obtained from three or more groups were determined by one-factor analysis of variance (ANOVA) followed by Tukey's post hoc test using GraphPad Prism5.0 software (GraphPad Software, San Diego, CA). $P < 0.05$ was considered statistically significant.

Results

Increased PPARα Expression in the Liver after PHx

PPARα controls β-oxidation in liver and serves as a potent hepatic mitogen in rodents.[39] To investigate the influence of PPARα on liver regeneration after PHx, PPARα and retinoid X receptor α protein expression was determined by Western blot analysis. PPARα protein level was increased at 12 and 32 hours after PHx (Figure 1, A and B). However, retinoid X receptor α protein levels were comparable between sham and PHx surgery (Supplemental Figure S1). To further assess the change of PPARα after PHx, *in vivo* luciferase assays were performed after PHx or sham at 32 hours. Luciferase activity was significantly increased after PHx surgery compared with sham-operated mice (Figure 1C). These results indicate that PPARα may be involved in PHx-induced liver regeneration.

Disruption of PPARα in Hepatocyte Inhibits Liver Regeneration after PHx

To further study the definite role of hepatocyte PPARα in PHx-induced liver regeneration, hepatocyte-specific PPARα-deficient ($Ppara^{\Delta Hep}$) mice were used.[33] The knockout efficiency was confirmed by real-time quantitative PCR and Western blot analysis (Supplemental Figure S2). When subjected to PHx, $Ppara^{\Delta Hep}$ mice exhibited significantly decreased mitosis in hepatocytes at 32 hours compared with $Ppara^{fl/fl}$ mice (Figure 2A). BrdU incorporation showed that peak hepatocyte proliferation appears at 40 hours after PHx in both $Ppara^{fl/fl}$ and $Ppara^{\Delta Hep}$ mice (Figure 2, B and C). However, $Ppara^{\Delta Hep}$ mice showed significantly reduced hepatocyte proliferation at 32 hours after PHx compared with $Ppara^{fl/fl}$ mice (Figure 2C). Consistently, the liver weight/body weight ratio after PHx also decreased in $Ppara^{\Delta Hep}$ mice (Figure 2D). Taken together, these results suggest that liver regeneration was impaired in $Ppara^{\Delta Hep}$ mice after PHx compared with $Ppara^{fl/fl}$ mice.

Disruption of PPARα in Hepatocytes Results in Decreased Cell Cyclin and DNA Repair Gene Expression after PHx

To further confirm whether hepatocyte PPARα deficiency inhibits the cell cycle after PHx, the kinetics of key cell cycle proteins, including CYCD1, CYCA2, CYCE1, and

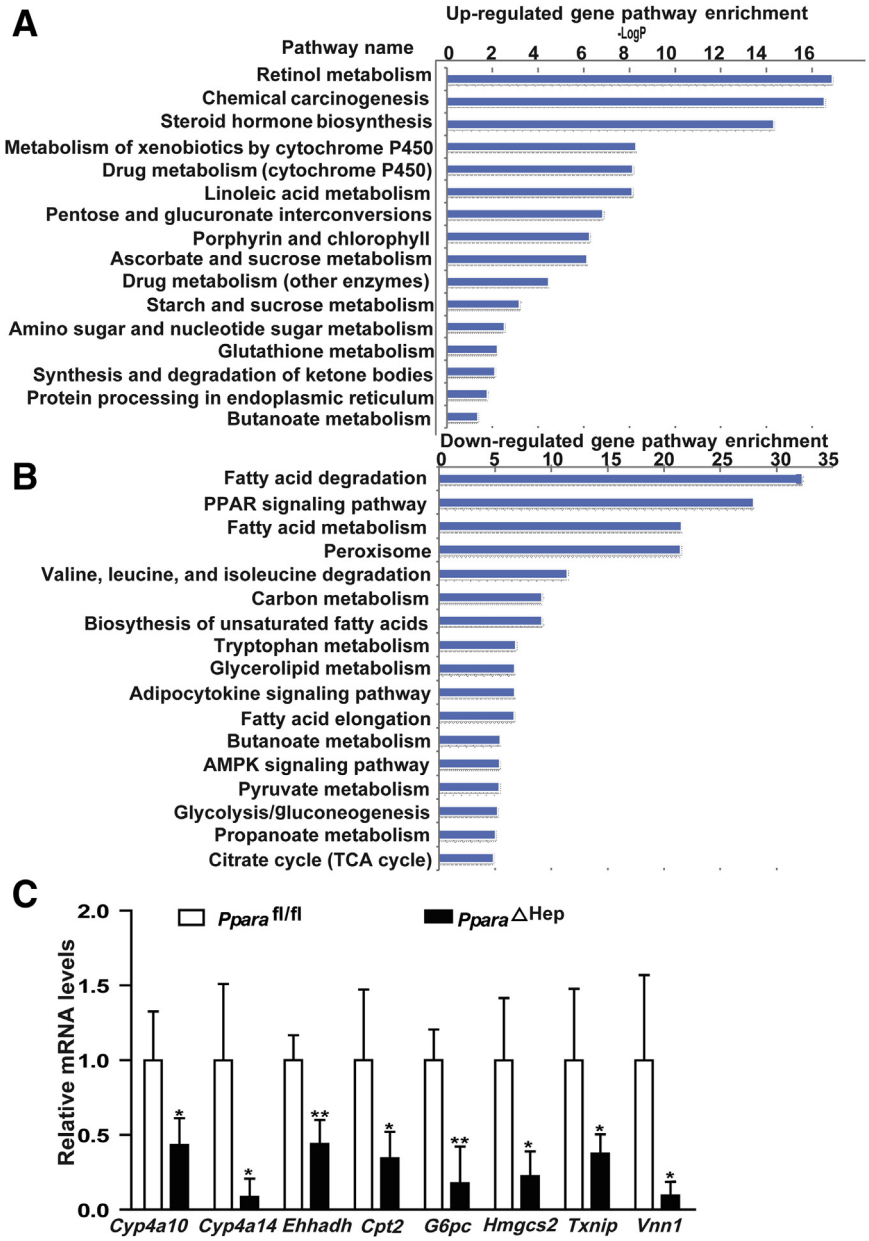

Figure 5 Microarray analyses for hepatic gene profiles from hepatocyte PPARα-deficient mice at 12 hours after PHx. **A:** Up-regulated gene pathways in *Ppara*$^{\Delta Hep}$ mice compared with control mice at 12 hours after PHx. **B:** Down-regulated gene pathways in *Ppara*$^{\Delta Hep}$ mice compared with *Ppara*$^{fl/fl}$ mice at 12 hours after PHx, as determined by microarray analysis. **C:** Quantification of *Cyp4a10*, *Cyp4a14*, *Cpt2*, *Ehhadh*, *G6pc*, *Hmgcs2*, *Txnip*, and *Vnn1* mRNAs in *Ppara*$^{\Delta Hep}$ and *Ppara*$^{fl/fl}$ mice. Data are expressed as means ± SD (**C**). $n = 4$ (**A**–**C**). $^{*}P < 0.05$, $^{**}P < 0.01$ versus *Ppara*$^{fl/fl}$. AMPK, adenosine 5′-monophosphate-activated protein kinase; TCA, tricarboxylic acid cycle.

PCNA expression, were measured. *Ccna2* and *Ccne1* mRNA levels were comparable between *Ppara*$^{fl/fl}$ and *Ppara*$^{\Delta Hep}$ mice; however, *Ccnd1* mRNA, expressed during G_1-S phase progression, was significantly decreased in *Ppara*$^{\Delta Hep}$ mice (Figure 3, A–C) after PHx compared with *Ppara*$^{fl/fl}$ mice, and *Pcna* mRNA levels also decreased at 32 hours after PHx compared with *Ppara*$^{fl/fl}$ mice (Figure 3D), consistent with BrdU incorporation assay (Figure 2). Finally, the CYCD1 and PCNA protein levels were determined in *Ppara*$^{fl/fl}$ and *Ppara*$^{\Delta Hep}$ mice after PHx at 0, 3, 6, 12, 24, and 32 hours. *Ppara*$^{\Delta Hep}$ mice showed a decrease of CYCD1 protein expression at different time points after PHx compared with that in *Ppara*$^{fl/fl}$ mice (Figure 3E). PCNA protein also decreased at 12, 24, and 32 hours after PHx in contrast with *Ppara*$^{fl/fl}$ mice (Figure 3F). These results suggest that hepatocyte PPARα may regulate cell proliferation by inducing cell cycle–related gene expression, which is in agreement with previous findings.[40]

It is well established that STAT3 and NF-κB signaling pathways are activated in the early stage of liver regeneration.[4] Activation of STAT3 and NF-κB was found at the early time points of 3 and 6 hours after PHx, as revealed by Western blot analyses (Supplemental Figure S3).[27] Surprisingly, the phosphorylated STAT3 level was slightly higher at 6 and 12 hours after PHx in *Ppara*$^{\Delta Hep}$ mice, and phosphorylated NF-κB was also higher at 0 and 3 hours after PHx in *Ppara*$^{\Delta Hep}$ mice when compared with that in *Ppara*$^{fl/fl}$ mice (Supplemental Figure S3). Activation of STAT3 and NF-κB was found to contribute to early liver regeneration after PHx.[4,9] Despite the slight increase in

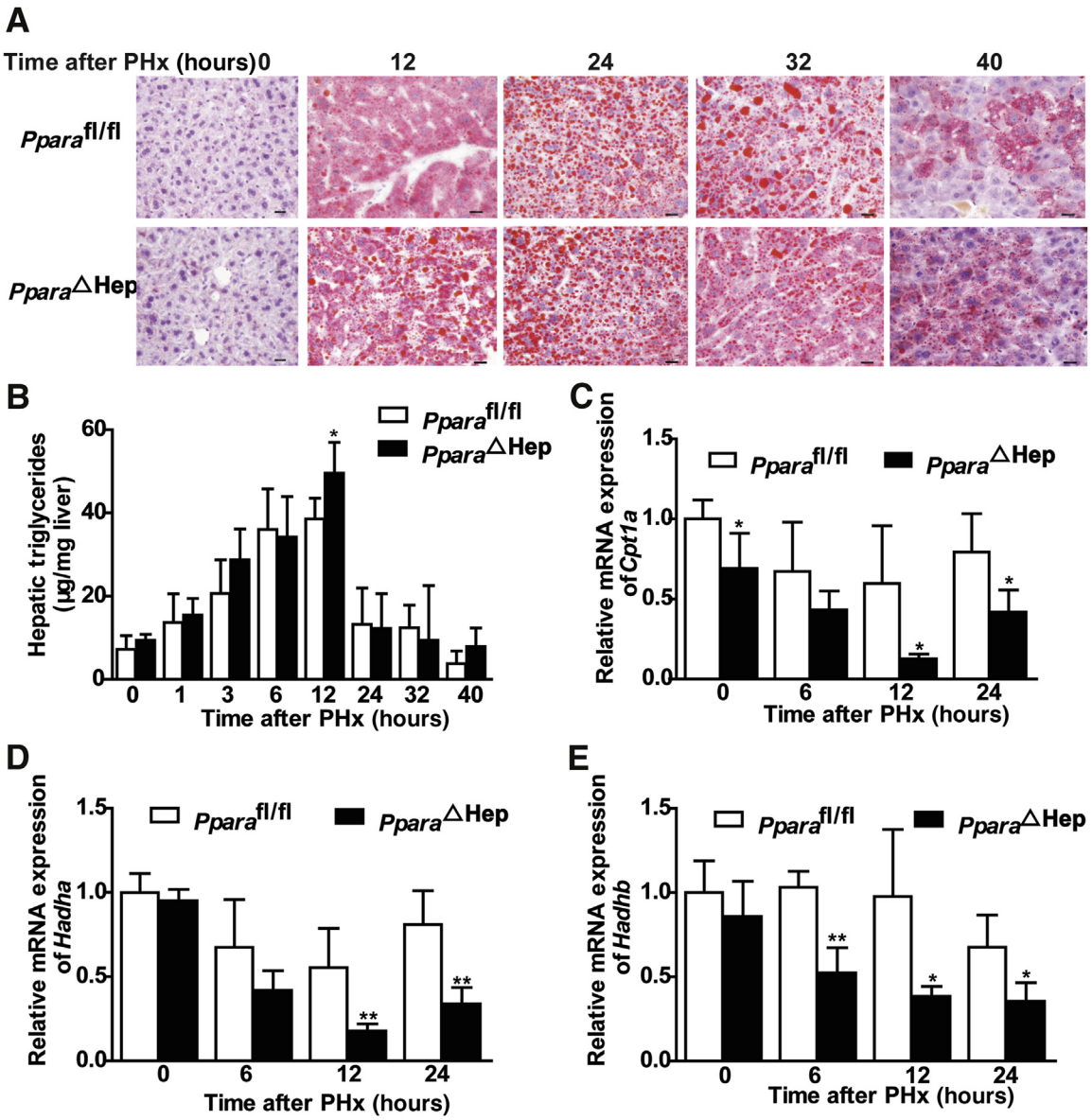

Figure 6 Hepatic PPARα deficiency promotes hepatic lipid accumulation during liver regeneration. **A:** Representative images for oil red O staining of liver tissues from *Ppara*$^{\Delta Hep}$ mice and *Ppara*$^{fl/fl}$ mice at 0, 12, 24, 32, and 40 hours. **B:** Hepatic triglyceride content in liver tissues. **C**–**E:** Fatty acid β-oxidation related *Cpt1a* and *Hadha/b* mRNAs. Data are expressed as means ± SD (**B**–**E**). $n = 5$ (**B**–**E**). $^{*}P < 0.05$, $^{**}P < 0.01$ versus *Ppara*$^{fl/fl}$. Scale bars = 20 μm (**A**). Original magnification, ×400 (**A**).

activation of STAT3 and NF-κB in the early stages of PHx, the decreased cell cycle–related protein expression in *Ppara*$^{\Delta Hep}$ mice suggested that the hepatocyte-specific PPARα-stimulated cell proliferation is STAT3/NF-κB independent.

Increased reactive oxygen species are found in liver after PHx, which could damage cellular DNA.[41] A previous study demonstrated that hepatic nonparenchymal cell DNA repair gene activation was dependent on PPARα.[42] Accordingly, hepatocyte PPARα may play a role in regulating DNA repair. DNA repair gene mRNA *Rad51*, *Prkdc*, and *Mcm2* were decreased at 24 hours in *Ppara*$^{\Delta Hep}$ mice when compared with that in *Ppara*$^{fl/fl}$ mice (Figure 4).

Microarray Analysis for Hepatic Gene Profile Changes from Hepatocyte PPARα-Deficient Mice at 12 Hours after PHx

PPARα acts as the master of fatty acid oxidation in liver; however, the changes of its target genes after PHx and the consequent effects on regenerative genes were unknown. At 12 to 24 hours after PHx, the mice develop marked steatosis.[11] Therefore, the time point of 12 hours after PHx was chosen to perform microarray analysis. Hepatic mRNA profiles were examined in *Ppara*$^{\Delta Hep}$ and *Ppara*$^{fl/fl}$ mice liver at 12 hours after PHx (Gene Expression Omnibus database, *https://www.*

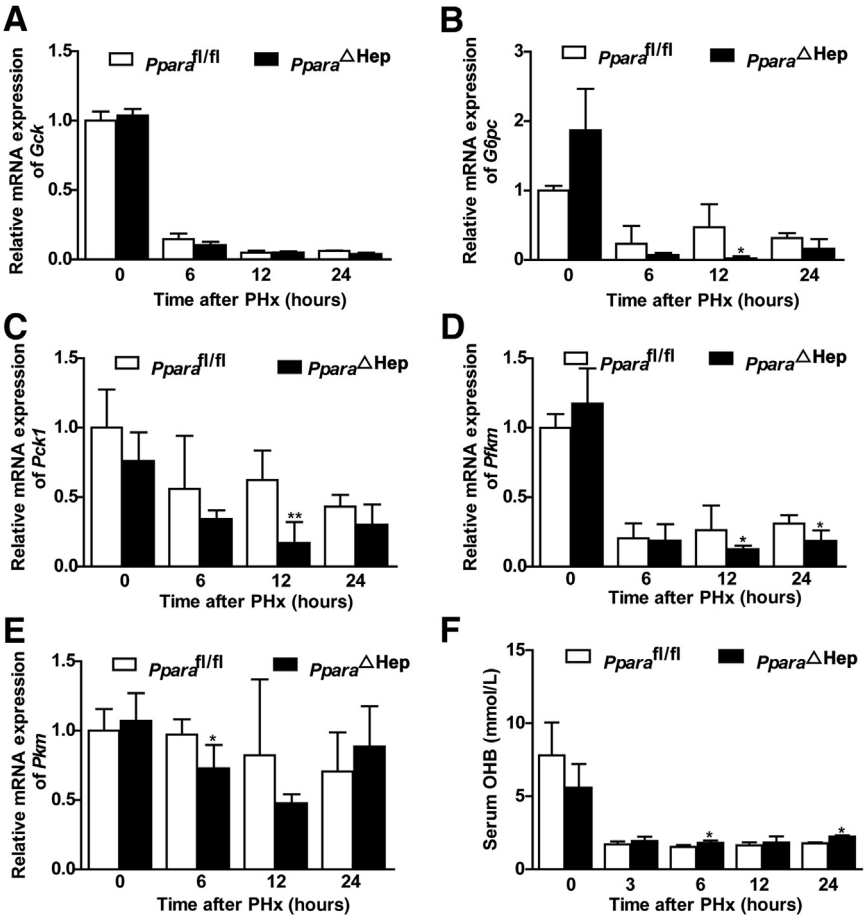

Figure 7 Hepatic PPARα deficiency reduces hepatic glucose metabolism gene expression during liver regeneration. **A**–**E:** mRNA expression of gluconeogenesis genes *G6pc* and *Pck1* (**A** and **B**) and glycolysis genes *Gck*, *Pkm*, and *Pfkm* (**C**–**E**). **F:** Serum hydroxybutyrate levels after PHx at 0, 3, 6, 12, and 24 hours in $Ppara^{\Delta Hep}$ and $Ppara^{fl/fl}$ mice. Data are expressed as means ± SD (**A**–**F**). $n = 5$ (**A**–**F**). $^{*}P < 0.05$, $^{**}P < 0.01$ versus $Ppara^{fl/fl}$.

ncbi.nlm.nih.gov/geo; accession number GSE114223). Pathway enrichment analysis showed that compared with control mice, the up-regulated genes in $Ppara^{\Delta Hep}$ mice are mainly involved in drug metabolism and detoxification and the cytochrome P450 family (Figure 5A). Because there were many down-regulated genes, only the top 20 pathway types were shown (Figure 5B). These genes were mainly involved in hepatic fatty acid metabolism, PPARα signaling, amino acid metabolism, glycolysis, and gluconeogenesis. The most obvious change was lipid metabolism (Figure 5B). To confirm this result, some of the mRNAs were quantitated, including *Cyp4a10/14*, *Cpt2*, *Ehhadh*, *G6pc*, *Hmgcs2*, *Txnip*, and *Vnn1*, revealing that they were lower, consistent with the microarray analysis (Figure 5C).

Hepatocyte PPARα Deficiency Promotes Hepatic Lipid Accumulation during Liver Regeneration

PPARα plays an important role in regulating fatty acid oxidation.[43] Microarray analysis results indicate that hepatic PPARα may promote liver regeneration after PHx by regulating the lipid metabolism to meet the energy requirement. To study the hepatic lipid metabolism changes after PHx, oil red O staining was used to measure the hepatic lipid accumulation. $Ppara^{\Delta Hep}$ mice showed more hepatic lipid accumulation and increased triglyceride contents than those in $Ppara^{fl/fl}$ mice at 12 hours after PHx (Figure 6, A and B). In accordance with this result, mRNAs encoded by genes involved in hepatic fatty acid β-oxidation, such as *Cpt1a* and *Hadha/b*, all decreased at 12 and 24 hours after PHx in $Ppara^{\Delta Hep}$ mice when compared with $Ppara^{fl/fl}$ mice (Figure 6, C–E). These results indicate that hepatic PPARα could promote liver regeneration by increasing fatty acid oxidation and lipid accumulation.

Hepatocyte PPARα Deficiency Reduces Hepatic Glucose Metabolism Gene Expression during Liver Regeneration

Previous studies showed that PPARα plays an essential role in maintaining glucose, lipid, and cholesterol homeostasis.[44] Moreover, glucose metabolism is a source of energy for liver regeneration.[11] Therefore, glycolysis and gluconeogenesis gene expression in liver after PHx were measured. *G6pc*, *Pck1*, *Pkm*, and *Pfkm* mRNA levels were decreased in $Ppara^{\Delta Hep}$ mice when compared with those in $Ppara^{fl/fl}$ mice (Figure 7,

A–E). These results show that PPARα may supply energy for liver regeneration by regulating glucose metabolism.

β-Hydroxybutyrate is a ketone body mainly produced in the liver from fatty acids during fasting, prolonged exercise, or absence of dietary carbohydrates, when the glucose supply is too low to meet the energy needs. β-Hydroxybutyrate is then distributed via the circulation to metabolically active tissues as a glucose-sparing energy source.[45,46] β-Hydroxybutyrate can promote histone hyperacetylation and reduce lipolysis and metabolic rates.[45] Hepatocyte-specific disruption of PPARα increased serum β-hydroxybutyrate and slightly decreased the metabolic rate at 6 and 24 hours after PHx (Figure 7F). Therefore, the energy supply was not enough for normal liver regeneration compared with *Ppara*$^{fl/fl}$ mice.

Discussion

In this study, hepatocyte PPARα was found to promote liver regeneration through regulating cell cycle and lipid/glucose metabolism. In addition to whole body PPARα's role in liver regeneration after PHx,[26,31,47] the current study revealed that PPARα expressed in the hepatocyte controls energy supplies to ensure normal liver cell cycle progression. A previous study showed that PPARα agonist induced hepatocyte proliferation mainly dependent on hepatocyte PPARα but not PPARα expressed in nonparenchymal cell in the liver.[33] Herein, more evidence is provided supporting a role for PPARα in hepatocyte in regulating cell proliferation in the PHx model.

PPARα is a ligand-activated nuclear receptor that participates in multiple pathophysiological processes, including lipid metabolism, glucose homeostasis, cell proliferation, and inflammatory response.[27] PPARα is mainly expressed in liver, heart, and kidney and to a lesser degree in other organs. Mice with global knockout of PPARα develop transiently impaired liver regeneration after PHx, which is associated with changes in expression of cell cycle control genes, cytokine signaling, and fat metabolism.[26] In the present study, conditional disruption of PPARα in hepatocytes resulted in a decreased liver regeneration after PHx. Moreover, in contrast with *Ppara*$^{fl/fl}$ mice, a decrease of *Ccnd1* mRNA and CYCD1 protein levels was noted at 12, 24, and 32 hours in *Ppara*$^{\Delta Hep}$ mice after PHx. CYCD1 is a key cell cycle protein that forms complexes with cyclin-dependent kinases 4 and 6, which promote cell cycle progression from the G_1 phase to the S phase.[48] Consistently, *Ppara*$^{\Delta Hep}$ mice showed decreased *Pcna* mRNA and PCNA protein expression at 32 hours after PHx. Furthermore, the present data indicate that the decreased CYCD1 and PCNA at the early stage of PHx in *Ppara*$^{\Delta Hep}$ mice is likely not because of activation of the STAT3/NF-κB pathway. These data suggest that hepatocyte PPARα promotes cell proliferation by regulating CYCD1 and PCNA expression.

Transient liver steatosis is found 12 to 24 hours after PHx and is critical for normal liver regeneration, but excess hepatic steatosis and disruption of hepatic lipid accumulation before PHx impair liver regeneration in mice.[19] A previous study reported that *Ppara* whole body knockout mice showed transiently reduced liver regeneration with decreased fatty acid β-oxidation.[26] To fully understand the relationship between liver regeneration and lipid metabolism in mice, microarray analysis was performed in *Ppara*$^{\Delta Hep}$ and *Ppara*$^{fl/fl}$ control mice after PHx, revealing decreased expression of mRNAs encoding hepatic fatty acid metabolism enzymes, PPARα signaling, amino acid metabolism, glycolysis, and gluconeogenesis gene expression in *Ppara*$^{\Delta Hep}$ mice. In support of these findings, hepatic lipid and triglyceride contents were increased, whereas fatty acid β-oxidation gene mRNAs were decreased in *Ppara*$^{\Delta Hep}$ mice compared with *Ppara*$^{fl/fl}$ mice at 12 hours after PHx. On the other hand, gluconeogenesis *G6pc*, *Pck1*, *Pkm*, and *Pfkm* mRNA levels were decreased in *Ppara*$^{\Delta Hep}$ mice compared with *Ppara*$^{fl/fl}$ mice, and serum hydroxybutyrate levels increased. These results suggest that hepatic PPARα promotes fatty acid β-oxidation, lipid metabolism, and glucose metabolism to meet the large energy requirement needed for liver regeneration.

In conclusion, the present study found that hepatocyte PPARα promotes liver regeneration after PHx at least partially via regulating cell cycle–related gene expression and promoting fatty acid β-oxidation and glucose metabolism.

Supplemental Data

Supplemental material for this article can be found at *https://doi.org/10.1016/j.ajpath.2018.10.009*.

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