Periostin promotes liver steatosis and hypertriglyceridemia through downregulation of PPARα

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Hepatosteatosis is characterized by an aberrant accumulation of triglycerides in the liver; however, the factors that drive obesity-induced fatty liver remain largely unknown. Here, we demonstrated that the secreted cell adhesion protein periostin is markedly upregulated in livers of obese rodents and humans. Notably, overexpression of periostin in the livers of WT mice promoted hepatic steatosis and hypertriglyceridemia. Conversely, both genetic ablation of periostin and administration of a periostin-neutralizing antibody dramatically improved hepatosteatosis and hypertriglyceridemia in obese mice. Overexpression of periostin resulted in reduced expression of peroxisome proliferator–activated receptor α (PPARα), a master regulator of fatty acid oxidation, and activation of the JNK signaling pathway. In mouse primary hepatocytes, inhibition of αβ4 integrin prevented activation of JNK and suppression of PPARα in response to periostin. Periostin-dependent activation of JNK resulted in activation of c-Jun, which prevented RORα binding and transactional activation at the Ppara promoter. Together, these results identify a periostin-dependent pathway that mediates obesity-induced hepatosteatosis.

Introduction

Triglycerides (TGs) are usually stored in adipose tissue as an energy source. However, aberrant TG accumulation in peripheral tissues, such as the liver, is one aspect of the metabolic syndrome and is associated with the development of type 2 diabetes, atherosclerosis, hypertension, and even coronary heart disease (1–3).

In humans, obesity is tightly associated with an increased risk of nonalcoholic fatty liver disease (NAFLD) (4). Hepatosteatosis occurs when TG homeostasis is disrupted, due to increased de novo lipogenesis and fatty acid uptake and reduced fatty acid oxidation and VLDL export (4, 5). However, the molecular mechanisms of obesity-induced fatty liver remain largely unknown.

Periostin (encoded by Postn) is a secreted cell adhesion protein belonging to the fasciclin family (6). Initially, periostin was thought to function as a homophilic adhesion molecule during bone formation and support osteoblastic cell attachment through binding to αvβ3, αvβ5, or α6β4 integrins (7). Analysis of Postn-null mice has revealed that periostin participates in the development of bone and tooth formation, acting on mesenchymal cells (6). Recently, increasing evidence also suggests that periostin is involved in the development of multiple tumors via several signaling pathways, such as PI3K/AKT and Wnt/?-catenin (8–11). In agreement with this, periostin expression is markedly upregulated in various human tumors, including in head and neck, breast, colon, pancreatic, and ovarian cancers (7, 12). However, the role of periostin in the regulation of TG homeostasis and in the pathogenesis of NAFLD remains uncharacterized.

In the present study, we identified a marked increase in periostin expression in the livers of obese mice and humans with fatty liver disease. Manipulation of periostin expression in the liver altered hepatic TG accumulation and serum TG levels.

Results

Hepatic periostin expression is upregulated in obese mice and humans with fatty liver disease. In order to identify genes that are potentially involved in dysfunctional hepatic lipid homeostasis in obesity, we previously performed a clustering analysis of Affymetrix arrays (13), which revealed that numerous mRNAs were markedly upregulated in the liver of mice fed a high-fat diet (HFD) compared with mice fed a normal chow diet (ND). In the liver of HFD-fed mice, expression levels of 7,198 genes were significantly changed (P < 0.05), of which 3,431 (47.7%) were increased and 3,767 (52.3%) were decreased. Our data showed a pronounced overexpression of periostin in the liver of HFD mice (Supplemental Table 1; supplemental material available online with this article; doi:10.1172/JCI74438DS1). Compared with that in primary nonhepatocytes isolated from control mice, periostin expression was 8-fold higher in mouse primary hepatocytes (MPHs; Supplemental Figure 1A), which indicates that hepatic parenchymal cells represent the main source of periostin expression. Increased Postn mRNA and periostin protein expression in HFD-fed mice was further confirmed by quantitative realtime RT-PCR (qRT-PCR) and ELISA, respectively (Figure 1A). To
The notion that the overproduction of periostin in the liver highlights an unanticipated and conserved feature of hepatosteatosis in obese rodents and humans.

Hepatic periostin expression is regulated by glucose. To clarify the physiological regulation of periostin in the liver, we investigated the effects of nutrients on periostin expression in vivo and in vitro. We used a continuous glucose infusion model as previously described (16). Infusion with 50% glucose (2 ml/h) via the jugular vein for 24 and 48 hours caused a sustained and significant increase in periostin expression in the liver of rats (Supplemental Figure 2A). Moreover, periostin levels were greater in the livers of C57BL/6 mice upon refeeding than in those fasted for 24 hours (Supplemental Figure 2B). We also examined Postn mRNA expression in cultured HepG2 or MPHs; addition of glucose at 15 and 25 mM dramatically increased Postn expression in a dose-dependent manner, whereas neither insulin, the cAMP agonist forskolin, nor the glucocorticoid analog dexamethasone changed Postn expression in these cells (Supplemental Figure 2, C and D).

Recent studies reported that the transcription factor carbohydrate-responsive element-binding protein (ChREBP) plays a
critical role in the induction of glucose-regulated genes in the liver (17, 18). Therefore, we used shRNA adenovirus to silence ChREBP in HepG2 cells, which resulted in a reduced response of perios- tin to high glucose (Supplemental Figure 3, A and B). We further generated a luciferase reporter construct containing the region of -1,037 bp to +10 bp of the mouse Postn promoter. The luciferase assay showed that the transcriptional activity containing this region was dramatically upregulated by ChREBP (Supplemental Figure 3C). By serial deletion within this region, we were able to define a potential ChREBP-binding site, located -356 bp to -340 bp upstream of the transcriptional start site (Supplemental Figure 3C). Moreover, mutation of this site largely abolished the regulation of ChREBP in periositin transcriptional activity (Supplemental Figure 3D), which indicates that this binding site is required for the induction of promoter activities by ChREBP. In addition, ChIP assays also showed that ChREBP was able to bind to this region of the Postn promoter in HepG2 cells exposed to high glucose (Supplemental Figure 3E). Thus, multiple lines of evidence suggest that increased periositin expression in the liver is due, at least in part, to overnutrition.

**Periostin promotes TG accumulation in the liver.** To elucidate the pathophysiological role of periositin in the liver, we overexpressed periositin in the liver of C57BL/6 mice by delivering an adenoviral vector with the Postn gene via tail vein injection (Supplemental Figure 4A). At day 10 after injection, overexpression of periositin resulted in a significant increase in TG content and liver weight (Figure 2, A and B). The prominent hepatosteatosis was also confirmed by H&E and Oil Red O staining (Figure 2C). In parallel, serum TG as well as serum VLDL fractionation was elevated (Figure 2, D and E). To determine the rate of VLDL secretion, we injected tyloxapol, an inhibitor of plasma lipases, into mice overexpressing periostin or GFP. Hepatic VLDL secretion was unaffected by periostin overexpression (Supplemental Figure 4B). Moreover, there was no significant change in BW, serum and hepatic cholesterol levels, or liver damage markers in periostin-overexpressing mice (Supplemental Figure 4, C–G).

We further treated HepG2 cells with 50 ng/ml recombinant periositin protein and found that the cellular TG content was dramatically increased (Figure 2F and Supplemental Figure 5A). Similar results were also obtained in MPHs (Figure 2G). The dose of periositin was chosen based on cell proliferation assays (Supplemental Figure 5B) and previous reports (8). Collectively, our results indicate that periositin promotes TG accumulation in the liver.
Figure 3. Periostin regulates liver lipid metabolism via PPARα. (A) Serum β-hydroxybutyrate levels in mice transduced with GFP or Postn adenoviruses (n = 6–7). (B and C) mRNA expression of hepatic Ppara (B) and protein expression of hepatic PPARα (C) in mice. (D) mRNA levels of hepatic Cpt1a, Mcad, and Acox1 in mice. (E) β-hydroxybutyrate levels in the medium of HepG2 cells. Cells were treated with periostin protein (50 ng/ml) or PBS vehicle control for 36 hours. (F) mRNA levels of Ppara, Cpt1a, Mcad, and Acox1 in HepG2 cells as cells in E. (G) Representative PPARα protein levels in HepG2 cells. (H) β-hydroxybutyrate levels in the medium of MPHs. Cells were treated with periostin protein (50 ng/ml) or PBS for 36 hours. (I) mRNA levels of Ppara, Cpt1a, Mcad, and Acox1 in MPHs as in H. (J) Representative PPARα protein levels in MPHs. (K) β3-palmitate oxidation rate in MPHs treated with periostin protein or PBS. MPHs were preincubated in maintenance medium for 24 hours in the presence of WY14443 (20 μM) or DMSO followed by incubation with 125 mM β3-palmitic acid and 1 mM carnitine in PBS for 2 hours. *H2O was then measured. n = 4. (L) Cellular TG content in MPHs isolated from WT or Ppara KO mice. Cells were treated with recombinant periostin protein (50 ng/ml) or PBS for 36 hours (n = 4–5). (M) mRNA levels of Cpt1a, Mcad, and Acox1 in MPHs. *P < 0.05, **P < 0.01, ***P < 0.001.

(20, 21), was particularly reduced (Figure 3, B and C). The MPHs isolated from Ppara KO mice were used as negative controls for PPARα Ab (Supplemental Figure 6A). Expression levels of the PPARα target genes carnitine palmitoyltransferase 1A (Cpt1a), medium-chain acylcoenzyme-A dehydrogenase (Mcad), and acyl-CoA oxidase 1, palmitoyl (Acox1), were also downregulated (Figure 3D), whereas genes involved in lipogenesis and lipid transport remained unaffected (Supplemental Figure 6B). In cultured HepG2 cells and MPHs, periostin treatment also led to decreased β-hydroxybutyrate in the culture medium and reduced expression of fatty acid oxidation–related genes (Figure 3, E–J, and Supplemental Figure 6, C and D). In addition, β-oxidation of 13-H-palmitate was significantly lower in MPHs treated with periostin than the vehicle control, even in the presence of the PPARα agonist WY14443 (Figure 3K). PPARα target genes were also downregulated by periostin in MPHs in the presence of WY14443 (Supplemental Figure 6E).

Next, we used MPHs isolated from Ppara KO mice. As expected, Ppara KO MPHs displayed an increase of TG content (Figure 3L). Notably, periostin increased cellular TG levels only in WT, not in Ppara KO, MPHs (Figure 3L). Consistent with this result, periostin reduced fatty acid oxidation–related genes only in WT MPHs (Figure 3M), underlying the role of periostin in suppressing PPARα function in this context.

Periostin downregulates PPARα through αβ4 integrin–mediated JNK activation. To explore the molecular mechanism by which periostin downregulates PPARα expression, we examined potential regulatory pathways. We found that periostin overexpression resulted in marked activation of the JNK signaling pathway, as JNK phosphorylation was increased, while the p38, ERK, and AKT pathways were unaffected (Figure 4A). In addition, phosphorylation of c-Jun, a downstream target of JNK kinase, was also enhanced by periostin overexpression (Figure 4A). The JNK pathway was also activated in HepG2 cells and MPHs treated with periostin (Figure 4, B and C).

Recent studies demonstrate that periostin can bind to the αβ3, αβ5, or αβ4 integrins (7). Our RT-PCR analysis showed that these integrins were expressed in the liver of C57BL/6 mice (Supplemental Figure 7A). To probe which of these integrins mediates periostin action, we preincubated MPHs with Abs against each pair of these integrins and measured the levels of phosphorylated JNK induced by periostin. Periostin activated the JNK pathway primarily through the αβ6 integrins, while the Abs against the αβ3 and αββ integrins did not block JNK phosphorylation (Figure 4D). Consistently, the Abs against the αβ6 integrins also attenuated the cellular TG content and restored periostin-suppressed Ppara expression levels (Figure 4, E and F).

To further investigate the role of JNK kinase activation in hepatosteatosis, we used 2 approaches to block JNK function and then examined the effect of periostin. First, mice overexpressing periostin by adenoviral Postn were treated with SP600125, a classic JNK kinase inhibitor (22). SP600125 abrogated the effect of periostin on liver weight and TG content in C57BL/6 mice (Supplemental Figure 7, B and C). The elevated serum TG and reduced serum β-hydroxybutyrate levels induced by periostin were also blocked by SP600125 (Supplemental Figure 7, D and E). Furthermore, the downregulation of PPARα and its target genes was also abrogated by SP600125 treatment (Supplemental Figure 7, F and G).

Additionally, MPHs isolated from WT and Jnk1 KO mice were used. Periostin treatment led to a dramatic increase in cellular TG content and decrease in fatty acid oxidation–related genes in WT MPHs, but not Jnk1 KO MPHs (Figure 4, G–I). Taken together, these data firmly establish that periostin promotes hepatosteatosis by activating the JNK pathway via αβ6 integrins.

Previous studies have shown that several integrins could activate Rac1 (23, 24), the upstream regulator of JNK signaling (25, 26). We explored whether periostin could activate Rac1 through αβ4 integrins. As evidenced by its translocation to the cell membrane, periostin definitely activated Rac1, which was attenuated by the Abs against αβ4 integrins (Supplemental Figure 8A). To further determine whether Rac1 is required for JNK activation by periostin, we pretreated HepG2 cells with the Rac1 antagonist NSC23766 (27), which largely abolished the JNK phosphorylation induced by periostin (Supplemental Figure 8B). Similar results were also obtained in HepG2 cells transfected with siRNA targeting Rac1 (Supplemental Figure 8C). Therefore, our results suggest that activation of JNK signaling by periostin and/or αβ4 integrins is dependent, at least in part, on Rac1.

To investigate the mechanisms of PPARα downregulation by JNK signaling, we examined the expression of several upstream regulators of PPARα, including Hnf4, Coup-TFII, and Car (28–30), which were not altered by periostin (Supplemental Figure 9A). In accordance with the regulation of PPARα expression, periostin inhibited Ppara promoter activity (Figure 5A). Using a series of truncated promoters, we defined a minimal periostin-responsive region (261 bp upstream of the transcriptional start site) that contained consensus binding sites for APE, CREB, SP1, and ROAR (Figure 5, A and B). Site-directed mutagenesis showed that the ROAR binding site (located between −57 bp and −40 bp) was indispensable for periostin inhibition of Ppara promoter activity (Figure 5, B and C). Indeed, treatment of HepG2 cells and MPHs with cholesterol sulfate (CS), a ROAR agonist (31), increased PPARα expression (Supplemental Figure 9B). Moreover, the luciferase activity of the Ppara promoter, which was dramatically enhanced by ROARs and CS, was largely abolished by mutation of this binding site (Supplemental Figure 9C). Therefore, these results suggest that ROARs may play a crucial role in mediating the periostin suppression of Ppara gene transcription.
The marked activation of c-Jun seen upon periostin treatment led us to investigate whether c-Jun played a role in the inhibition of Ppara gene transcription. In transient transfection assays using the Ppara-luciferase reporter (–261 Luc) in HepG2 cells, overexpression of c-Jun markedly suppressed the Ppara reporter activity induced by RORα, in the absence and presence of CS (Figure 5C). However, mutations in the RORα binding site significantly prevented the c-Jun suppression of the Ppara reporter (Figure 5C), which suggests that c-Jun could prevent the RORα activation of PPARα and result in inhibition of Ppara gene transcription. Since c-Jun inhibited RORα-induced Ppara gene transcription, we postulated that these 2 molecules might interact with each other. Indeed, co-IP assays in

Figure 4. Periostin activates the JNK signaling pathway to promote hepatosteatosis. (A) Phosphorylated and total JNK, c-Jun, p38, AKT, and ERK in the liver of C57BL/6 mice injected with GFP or Postn adenovirus. Total JNK, c-Jun, p38, AKT, and ERK were used as loading controls. (B and C) Phosphorylated and total JNK and c-Jun in HepG2 cells (B) and MPHs (C) incubated with PBS or periostin protein for 1 hour. (D) Phosphorylated and total JNK in MPHs incubated with PBS or periostin protein. Cells were preincubated with Abs against the indicated integrins or IgG as a control for 2 hours, and then treated with PBS or periostin protein for another 1 hour. (E) Cellular TG content in MPHs incubated with PBS or periostin protein. Cells were preincubated with Abs against integrins or IgG control for 2 hours, then treated with PBS or periostin protein for another 36 hours. (F) Ppara mRNA levels in MPHs incubated with PBS or periostin protein. Cells were preincubated with Abs against integrins or IgG control for 2 hours, then treated with PBS or periostin protein for another 24 hours. (G) Cellular TG content in MPHs isolated from WT and Jnk1 KO mice. Cells were treated with periostin protein (50 ng/ml) or PBS for 36 hours (n = 4). (H and I) mRNA levels of Ppara (H) and its target genes (I) in MPHs. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 5. Periostin inhibits PPARα expression through c-Jun–mediated suppression of RORα transcriptional activity. (A) Luciferase reporters carrying a series of truncated mouse Ppara promoter. The promoter region from −1,242 to +1 bp was cloned and transfected into HepG2 cells. The transcription start site was set as +1 bp. (B) WT and mutant (Mut) Ppara promoters (−216 to +1 bp) were transfected into HepG2 cells, and luciferase reporter assays were measured. Potential binding sites for ATF/CREB, SP1, and RORα were mutated as indicated. (C) WT and RORα binding site mutant Ppara promoters (−216 to +1 bp) were cotransfected with RORα, c-Jun, or empty vectors into HepG2 cells. Cells were treated with CS (5 μM) or DMSO vehicle control for 12 hours before harvest. (D) RORα interacted with c-Jun. c-Jun was pulled down by RORα by IP in HEK293T cells transfected with Flag-tagged RORα and HA-tagged c-Jun. (E) Interaction of endogenous RORα and c-Jun. Cell lysates were extracted from HepG2 cells and subjected to IP using c-Jun or IgG Ab and IB using RORα Ab. (F) ChIP analysis showing binding of RORα and acetylated histone H3 (Ac-H3) to the Ppara promoter. MPHs were preincubated with IgG or Abs targeting α6β4 integrins for 2 hours, then treated with PBS or periostin for another 2 hours. The exon 1 region of Ppara was used as a negative control. **P < 0.01, ***P < 0.001.
HEK293T and HepG2 cells demonstrated that c-Jun could interact with RORα (Figure 5, D and E). Furthermore, ChIP assays demonstrated that the amount of RORα protein bound to the Ppara promoter was significantly reduced in MPHs treated with periostin, which could be prevented by α6β4 Abs (Figure 5F). Moreover, acetylated histone H3, a transcribed gene marker, exhibited changes similar to those observed with RORα (Figure 5F). Taken together, our data indicate that c-Jun may block RORα binding to the Ppara promoter, resulting in the inhibition of Ppara gene transcription.

**Disruption of Postn improves hepatosteatosis in obese mice.** To determine whether knockdown of endogenous Postn improves hepatosteatosis, we disrupted its expression in the liver of db/db mice by delivering 2 independent adenoviruses expressing Postn-specific shRNA or adenovirus expressing a nonspecific control shRNA. Postn shRNA treatment significantly reduced hepatic Postn mRNA and periostin protein levels compared with negative control shRNA–injected littermates (Figure 6A). shRNA-mediated loss of hepatic Postn dramatically reduced hepatic TG content, improved hepatosteatosis and serum TG levels, and increased circulating β-hydroxybutyrate (E) levels in db/db mice. (F) Phosphorylated JNK and c-Jun were determined in db/db mice with Postn knockdown or controls. (G) mRNA levels of fatty acid oxidation–related genes in mice as in F. *P < 0.05, **P < 0.01, ***P < 0.001.
partate aminotransferase (AST) (Supplemental Figure 11, A–F). At the molecular level, silencing the Postn gene attenuated the activation of JNK pathways and upregulated fatty acid oxidation–related genes (Figure 6, F and G, and Supplemental Figure 11G).

To investigate the long-term effect of periostin deficiency on hepatosteatosis, we used Postn-heterozygous mice, since Postn homozygotes exhibit postnatal growth retardation and skeletal defects (6, 7). Postn-heterozygous mice showed a 58% reduction in Postn mRNA and a 41% reduction in periostin protein in the liver (Supplemental Figure 12, A and B). WT and Postn-heterozygous mice at the age of 7 weeks were fed HFD for another 8 weeks. HFD caused similar levels of obesity in the 2 groups, as shown by BW, food intake, and fat content (Supplemental Figure 12, C–E). However, liver weight and TG content were significantly reduced in Postn-heterozygous mice (Figure 7, A–C). Serum TG levels and VLDL fraction were also markedly reduced, whereas circulating β-hydroxybutyrate levels were elevated, in Postn-heterozygous mice (Figure 7, D–F). Consistent with these results, JNK signaling activation was attenuated and fatty acid oxidation–related genes were upregulated in Postn-heterozygous mice (Figure 7, G and H). Conversely, serum cholesterol levels and liver cholesterol content, circulating fatty acids, and liver enzymes were comparable between groups (Supplemental Figure 12, F–I).

Neutralization of periostin improves fatty liver and hypertriglyceridemia in obese mice. Finally, we developed a neutralizing Ab to interfere with periostin and administered it to db/db mice. The specificity and efficiency of the neutralizing Ab were shown by Western blot and ELISA (Supplemental Figure 13, A and B). In mice treated with periostin neutralizing Abs, liver weight, hepatic TG
content, and serum TG levels were markedly reduced compared with IgG controls, whereas β-hydroxybutyrate levels were higher (Figure 8, A–D). Fatty acid oxidation–related genes were also upregulated (Figure 8E). Additionally, JNK signaling activation was alleviated, as shown by reduced phosphorylated JNK (Supplemental Figure 13C). However, BW was unchanged (Figure 8F). Taken together, these results indicate that blocking periostin with a neutralizing Ab can improve hepatosteatosis and hypertriglyceridemia in obese mice.

Discussion

Our present findings show that aberrant expression of periostin in the liver results in steatosis and hypertriglyceridemia through JNK-mediated suppression of fatty acid oxidation (Figure 8G). Nutrients, including high glucose concentrations, can upregulate periostin expression. Although glucose-induced repression of fatty acid oxidation has been previously studied (32, 33), the underlying molecular pathway is poorly understood. Therefore, these results also reveal a novel mechanism clarifying how glucose can regulate hepatic lipid metabolism. We also demonstrated that palmitate could increase periostin expression in HepG2 cells and MPHs (Supplemental Figure 14), which suggests that fatty acids might also be involved in regulation of periostin expression in obesity–associated hepatosteatosis.

It has been reported that periostin exerts its biological functions through several signaling pathways in the development of human cancers, including PI3K/AKT and Wnt/β-catenin (12, 34, 35). However, AKT phosphorylation was not altered in livers of mice overexpressing periostin. Indeed, a large portion of periostin’s effects in hepatic lipid management seems to depend on JNK activation, which suggests that this kinase represents a main mechanistic checkpoint for periostin action in the liver. Although this inconsistency in mechanism for cancer and metabolic disorders currently remains unexplored, the downstream signaling activated by periostin might be cell or tissue specific. In addition, studies using genetically engineered mice have shown that hyperactivation of the JNK pathway contributes to the development of metabolic diseases, including hepatic steatosis, obesity, and insulin resistance (36–38). Consistent with this, strong activation of JNK has been observed in the livers of HFD-fed mice and genetically obese mice (39, 40). Interestingly, only JNK1 deficiency has been shown to attenuate hepatocyte damage and steatosis, while...
JNK2 deficiency actually increased liver injury (41), showing the different roles of 2 JNKs in hepatic lipid metabolism (42).

Our results indicate that hepatic peristin overproduction phenocopies PPARα deficiency with respect to TG levels, and loss of PPARα was sufficient to significantly blunt peristin’s effects. It has been well established that disorders of hepatic fatty acid oxidation, including deficiency in PPARs or its coactivators, lead to massive hepatic TG accumulation and hypertriglyceridemia in animals (43–47). Moreover, some emerging evidence has shown that hepatic fatty acid oxidation is also impaired in human NAFLD. For instance, it was reported that variants in Ppara or Pgclα, a coactivator of PPARs, were significantly associated with NAFLD (48, 49). Deficiency of those genes involved in fatty acid oxidation, such as MCAD, long-chain acyl-CoA dehydrogenase (LCAD), and long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), were also shown to affect the development of human NAFLD (50, 51). In addition, some studies revealed that PPARα expression was downregulated by 50% in human NAFLD (52, 53). Consistently, our data showed that serum β-hydroxybutyrate levels were significantly reduced in NAFLD patients (Supplemental Figure 15, A–D). Moreover, hepatic TG content was inversely correlated with serum β-hydroxybutyrate level (Supplemental Figure 15, E–G). Previous studies have shown that the L162V variant of PPARα was significantly associated with several components of the metabolic syndrome, including fasting hypertriglyceridemia (54–56). We also found that obese subjects with the PPARα L162V genotype had a higher hepatic TG content (Supplemental Figure 16). Taken together, these results suggest that PPARα or fatty acid oxidation disorders may also participate in the development of human NAFLD. In addition, some studies have demonstrated that PPARα agonist improved liver steatosis in several obese mice models (57–59). However, the effect of PPARα agonists on improving human NAFLD is still controversial, although some studies have shown positive effects (60, 61). Therefore, large, long-term prospective clinical studies are still needed to fully assess the effects of PPARα agonists.

In agreement with our observations in the liver, suppression of Ppara transcription by JNK has also been shown in the heart (62), although the molecular mechanism remains largely unexplored. We identified a putative binding motif for RORα in the proximal promoter region of Ppara. It was reported that RORα regulated lipid homeostasis in skeletal muscle through upregulation of caveolin-3 and CPT-1α (63). Additionally, activation or overexpression of RORα attenuated hepatosteatosis through AMPK-mediated inhibition of Lxrα transcriptional activity (31). Therefore, our present results reveal an additional mechanism that reinforces the effect of RORα on hepatic lipid metabolism. The perturbed function of RORα by JNK/c-Jun contributed to the peristin-inhibiting effect of Ppara transcription. Co-IP studies in HEK293T and HepG2 cells uncovered an interaction between c-Jun and RORα, by which c-Jun blocked the recruitment of RORα to the Ppara promoter region (Figure 5, D and E). It has previously been shown that c-Jun participated in active repression of several nuclear transcription factors, such as MyoD, SMAD3, and HNF1α (64–66). However, further studies are still required to establish the precise mechanisms, which may involve the recruitment of transcriptional corepressors or other alternative linker proteins.

Recent studies have demonstrated that the liver can produce several proteins and secrete them into the circulation, where they participate in the regulation of hepatic and systemic glucose and lipid metabolism. These include fibroblast growth factor 21 (FGF21), α2-HS-glycoprotein (Fetuin-A), and pancreatic-derived factor (PANDER) (67–69). Intriguingly, the dysregulation of those hepatokines is closely associated with hepatosteatosis and dyslipidemia (68–71). Our present results indicate that peristin can also act as a hepatokine to regulate lipid metabolism in the liver. However, whether hepatic peristin has endocrine effects on distant targets, including adipose tissues or skeletal muscles (Figure 8G), awaits further in-depth analysis in future studies.

Together, our findings provided novel insight into the regulation of hepatic TG homeostasis and the mechanism of hepatosteatosis in obese rodents and humans. We identified peristin as an important regulatory molecule in the development of fatty liver. Peristin might therefore be a therapeutic target for fatty liver and dyslipidemia.

**Methods**

Further information can be found in Supplemental Methods.

**Animal experiments.** Male C57BL/6, ob/ob, and db/db mice aged 8–12 weeks were purchased from the Shanghai Laboratory Animal Company (SLAC). Postn and Ppara KO mice were obtained from Jackson Laboratories. Jnk1 KO mice were provided by G. Chen (Shanghai Jiao Tong University School of Medicine). All mice were housed at 21°C ± 1°C with humidity of 55% ± 10% and a 12-hour light/12-hour dark cycle. HFD-induced obese mice were maintained with free access to high-fat chow (D12492; Research Diets) and drinking water. HFD contained 60% kcal from fat, 20% kcal from carbohydrate, and 20% kcal from protein. ND contained 10% kcal from fat, 70% kcal from carbohydrate, and 20% kcal from protein.

**Human subjects.** In total, 1,043 adult participants were enrolled in a human cross-sectional study from April to August 2011, as described previously (15). Subjects were screened with physical examination and type B ultrasonography for fatty liver in the Lian Qian community (Xiamen, China). All participants completed a standard questionnaire including physical activity, diet, and history of present and past illnesses and medications. Anthropometric measurements included BW, height, waist circumference, blood pressure (BP), BMI, and body fat. BMI was calculated as weight (in kilograms) divided by the square of the height (in meters). Waist circumference was measured at the midpoint between the inferior costal margin and the superior border of the iliac crest on the midaxillary line. Those who drank 140 or 70 g/week of alcohol (for men or women, respectively), at the time or in the previous 6 months, were excluded from the study. HBV- or HCV-infected subjects were excluded. For analysis of serum peristin levels, blood samples were collected from 135 NAFLD patients with TG content >20% and 105 aged-matched normal subjects with hepatic TG content <5%, defined using Proton Magnetic Resonance Spectroscopy (1H-MRS) (15). For analysis of hepatic peristin concentrations and TG contents, liver biopsy was performed in those subjects who donated their partial livers for liver transplantation. The subjects were screened with physical examination and type B ultrasonography. H&E staining was performed for histology analysis.

**Cell culture.** HepG2 cell lines were purchased from the Cell Bank of Type Culture Collection, Chinese Academy of Sciences (CAS), and maintained in DMEM (Gibco) supplemented with 10% FBS (Gibco), 100 IU/ml penicillin, and 100 μg/ml streptomycin. MPHs were isolated from adult C57BL/6 mice (8–10 weeks of age) by collagenase perfusion and purified by centrifugation. Freshly prepared hepatocytes were
seeded at a final density of 0.5 × 10⁴ cells/well in 6-well plates in attachment media (ScienCell). The media were replaced with DMEM (Gibco) at 24 hours. Periostin recombinant protein was purchased from Abcam.

**Hepatic and cellular TG measurement.** Liver tissues were homogenized in chloroform/methanol (2:1 v/v) using a Polytron tissue grinder (Kinematica AG). Lipid extracts were prepared by the classical Folch method. Extracts were dried under N₂ flow and dissolved in isopropanol. For the in vitro model of cellular steatosis, HepG2 cells or MPHs were exposed to periostin protein (50 ng/ml; Abcam) or PBS vehicle control. TG and cholesterol contents were measured using commercial kits (Biovision) according to the manufacturer’s instructions.

**Fast protein liquid chromatography.** Serum from 5 mice per experimental group was pooled and subjected to fast protein liquid chromatography. VLDL and TG was measured in the eluted fractions using commercial kits (Biovision).

**Microarray analysis and qRT-PCR.** Affymetrix array hybridization and scanning using Mouse Genome 430 2.0 chips (Gene Tech Co. Ltd.) has been previously described (13). The full datasets were deposited in GEO (accession no. GSE57425). In order to quantify the transcripts of the genes of interest, qRT-PCR was performed using SYBR Green Premix Ex Taq (TaKaRa) on a Light Cycler 480 (Roche). See Supplemental Methods for primer sequences.

**Western blot.** Hepatic tissues or cells were lysed in radiomunoprecipitation buffer containing 50 mM Tris-HCl, 150 mM NaCl, 5 mM MgCl₂, 2 mM EDTA, 1 mM NaF, 1% NP-40, and 0.1% sodium dodecyl sulfate. Western blot was performed using Abs against ChREBP (ab157153; Abcam), periostin (SAB4300696; Sigma-Aldrich), PPARα (MAB8390; Millipore), JNK (4668, 9258; Cell Signaling), c-Jun (2939, 9163; Cell Signaling), p38 (9215, 2387; Cell Signaling), AKT (MAB3890; Millipore), JNK (4668, 9258; Cell Signaling), c-Jun (2939, 9163; Cell Signaling), and GAPDH (5174; Cell Signaling).

**Periostin neutralizing Ab.** The hybridoma cell lines secreting mouse mAb against mouse periostin were generated by Abmart Co., according to the standard hybridoma technique (72). The mouse mAbs were purified by Protein A affinity chromatography. mAb purity was confirmed by HPLC. The concentrations of the obtained mAbs were measured by Mouse IgG ELISA Quantitation kit (Bethyl Laboratories), following the manufacturer’s instructions. The kinetic parameters of the mAbs were determined using a Biacore T100 instrument (Biacore AB). Purified Ab was diluted in saline and injected at a dose of 5 mg/kg into db/db mice for 2 weeks.

**Statistics.** All values are shown as mean ± SEM. Statistical differences were determined by 2-way ANOVA with Bonferroni-adjusted post-test or by 2-tailed Student’s t test. A P value less than 0.05 was considered significant.

**Study approval.** The animal protocol was reviewed and approved by the Animal Care Committee of Shanghai Jiao Tong University School of Medicine. The human study was approved by the Human Research Ethics Committee of the Xiamen First Hospital, Xiamen University (Xiamen, China) and the Ethics Committee of Shanghai Jiao Tong University School of Medicine. Written informed consent was obtained from each subject obtained was obtained.

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30. Maglich JM, Lobe DC, Moore JT. The nuclear orphan receptor αv/b1 Integrin activates Rac1 in gene transcription by JNK-mediated phosphorylation.
57. Li J, et al. Pancratic-derived factor promotes hepatic fibroblast growth factor 21 expression and attenuates hepatic steatosis despite reducing plasma triglyceride levels.