Arch Toxicol (2015) 89:1569–1578
DOI 10.1007/s00204-014-1322-7

MOLECULAR TOXICOLOGY

Activation of sterol regulatory element-binding proteins in mice exposed to perfluorooctanoic acid for 28 days

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Received: 10 April 2014 / Accepted: 21 July 2014 / Published online: 6 August 2014

Abstract Perfluoroalkyl acids are widely used in numerous industrial and commercial applications due to their unique physical and chemical characteristics. Although perfluorooctanoic acid (PFOA) is associated with hepatomegaly through peroxisome proliferator-activated receptor α (PPARα) activation, liver fat accumulation and changes in gene expression related to fatty acid metabolism could still be found in PPARα-null mice exposed to PFOA. To explore the potential effects of PFOA on sterol regulatory element-binding proteins (SREBPs) activity, male mice were dosed with either Milli-Q water or PFOA at doses of 0.08, 0.31, 1.25, 5, and 20 mg/kg/day by gavage for 28 days. Liver total cholesterol concentrations and PFOA contents showed a dose-dependent decrease and increase, respectively. Transcriptional activity of PPARα and SREBPs was significantly enhanced in livers. Protein expression analyzed by Western blotting showed that PFOA exposure stimulated SREBP maturation. Furthermore, proteins blocked SREBP precursor transport, insulin-induced gene 1 (INSIG1) and INSIG2 proteins, as well as a protein-mediated nuclear SREBP proteolysis, F-box and WD-40 domain protein 7, decreased in mouse liver exposed to PFOA. The expression levels of the miR-183-96-182 cluster, which is possibly involved in a regulatory loop intermediated by SREBPs maturation, were also increased in the mouse liver after PFOA exposure. We also observed that PFOA induced lipid content and PPARα in Hepa 1-6 cells after exposure to PFOA for 72 h but SREBPs were not activated in vitro. These results demonstrated that SREBPs were maturated by activating the miR-183-96-182 cluster-SREBP regulatory loop in PFOA-exposed mouse liver.

Keywords SREBP · Lipid metabolism · PPAR · Perfluorononanoic acid

Introduction

Perfluoroalkyl acids (PFAAs) are a family of anthropogenic compounds widely used in industrial and commercial applications due to their unique physical and chemical characteristics (Lau et al. 2007). The toxicological effects of two of the most widely known PFAAs, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), have been extensively studied, and earlier reports have showed PFOA and PFOS to have similar effects in rodents, such as reduced body weight, increased liver weight, reduced serum cholesterol, and dose-dependent increased mortality (Lau et al. 2007).

Among phenomena induced by PFOA and PFOS, liver enlargement in rodents is evident even at low-dose exposure (Wan et al. 2012; Yan et al. 2014). Several studies have determined the reasons of PFAA-induced hepatomegaly and have shown that PFOA and PFOS are capable of inducing peroxisome proliferation, with activation of peroxisome proliferator-activated receptors (PPARs) the first key event (Lau et al. 2007; Takacs and Abbott 2007). PPARs are a group of closely related ligand-dependent nuclear receptors that mediate the effects of eicosanoids, fatty acids, and synthetic peroxisome proliferators on gene transcription (Kersten et al. 2000). Three PPAR isotypes, peroxisome

Electronic supplementary material The online version of this article (doi:10.1007/s00204-014-1322-7) contains supplementary material, which is available to authorized users.

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proliferator-activated receptor α (PPARα), β/δ and γ, have been identified in various tissues encoded by separate genes and play specific roles in controlling lipid metabolism and inflammation (Kersten et al. 2000; Takacs and Abbott 2007; Varga et al. 2011). Consistent with their master regulatory functions in metabolism, PPARs are highly expressed in tissues most active in lipid metabolism, with PPARα highly expressed in the liver (Varga et al. 2011). PPARα plays an important role in fatty acids β-oxidation, ketogenesis, and systemic lipid metabolism, and PPAR agonists reportedly induce hepatomegaly and hepatocarcinogenesis without genetic damage (Pyper et al. 2010). Although PFOA- and PFOS-induced liver enlargement appears to result from acting as a PPAR ligand, liver weight increase was still observed in PFOA-exposed PPARα-null mice (Minata et al. 2010; Wolf et al. 2008). Fat accumulation and changes in genes related to fatty acid metabolism were also observed in PFOA-treated PPARα-null mice (Minata et al. 2010; Rosen et al. 2008a, b), indicating that the activation of other alternate nuclear receptors may also be involved in lipid metabolism disturbance induced by PFOA. Several potential targets of PFOA and PFOS toxicity, which may be independent or indirectly controlled via cross talk with PPARα, were previously analyzed in vitro and seem to be activated in cells of rats more so than that of humans after exposure to PFOA or PFOS (Bjork et al. 2011).

The sterol regulatory element-binding proteins (SREBPs), a family of membrane-bound, basic helix-loop-helix leucine zipper (bHLH-LZ) transcription factors, play a critical role in controlling cholesterol and lipid metabolism and are involved in several biological processes and disease states (Bengoechea-Alonso and Ericsson 2007; Shao and Espenshade 2012). Three major SREBP proteins (SREBP1a, SREBP1c, and SREBP2) are encoded by the *Srebf1* and *Srebf2* genes, and most data suggest that the regulatory functions of the two SREBP1 isoforms are primarily in fatty acid metabolism and SREBP2 is mainly in cholesterol metabolism (Jeon and Osborne 2012; Shao and Espenshade 2012). Tissue distribution of the three mammalian SREBPs differs, with SREBP1c the predominant isoform in most adult nondividing metabolic tissues such as liver and adipose (Jeon and Osborne 2012; Raghow et al. 2008). SREBPs are translated as inactive precursors and activated by a set of elaborate maturation pathways, which are regulated by multiple signals. Sterol, insulin, and insulin-induced gene (INSIGs) play a key role in sterol-dependent regulation of SREBP (Shao and Espenshade 2012). Not only is the maturation pathway of SREBPs adjusted under different conditions, nuclear SREBPs are also highly regulated by posttranslational modification, such as phosphorylation, acetylation, and ubiquitinylation (Bengoechea-Alonso and Ericsson 2007).

Earlier reports showed a complex cross talk between PPARs and SREBP (Fernandez-Alvarez et al. 2011; Gao et al. 2013; Knight et al. 2005; Yoshikawa et al. 2003) in vivo and in vitro. However, the exact mechanism of the cross talk between PPARs and SREBPs is still unclear, especially the inconsistent results in vitro treated with WY-14,643 or PPARα and γ over expression. The induction of SREBP1c gene expression at the transcriptional level by PFAAs has been reported before (Bjork et al. 2011; Fang et al. 2012), but whether SREBPs play a role in effects induced by PFAAs exposure on the liver is still unclear. In the present study, we assessed the activation of SREBPs, especially key factors in the maturation pathway of SREBPs, in vivo and in vitro after PFOA exposure. We found that induction of the maturation pathway was involved in SREBPs activation after PFOA exposure.

Materials and methods

Animal treatment

Male Balb/c mice (age 6–8 weeks) were obtained from the Weitong Lihua Experimental Animal Center (Beijing, China), and all experimental manipulations are described in our previous study (Yan et al. 2014). In brief, mice were randomly divided into six groups and dosed with either Milli-Q water or PFOA diluted in Milli-Q water at doses of 0.08, 0.31, 1.25, 5, and 20 mg/kg/day by gavage for 28 days. The doses of PFOA were chosen according to earlier studies and our previous experiments. All animal treatments were approved by the Committee on the Ethics of Animal Experiments from the Institute of Zoology, Chinese Academy of Sciences (Permit Number: EET-015-08-2012).

Liver PFOA determination

PFOA was extracted from the livers of each group ($n = 3$) and analyzed using high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS).

Cell culture

The mouse hepatocellular carcinoma cell line Hepa 1-6 (Cell Resource Center, IBMS, CAMS/PUMC) was used to determine the effects of PFOA and WY-14,643 (a PPARα ligand) in vitro. The doses of each chemical we chose were based on our previous cell viability test using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma–Aldrich, Steinheim, Germany) method (Fig. S1 and S2).

Determination of TCHO and TG concentrations

Tissue total cholesterol and tissue triglyceride assay kits (Applygen Technologies, Beijing, China) were used for

determination of liver total cholesterol (TCHO) and TG concentrations, respectively.

Detection of neutral lipids change in cells

Neutral lipid changes in Hepa 1-6 cells after exposure to PFOA were detected by flow cytometry using fluorescent neutral lipid dye BODIPY 493/503 (Molecular Probes, Carlsbad, CA, USA).

Real-time PCR analysis

Total RNAs were isolated from the mice livers ($n = 6$) and Hepa 1-6 cells using TRIzol (Life Technologies-Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and were measured by a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA). cDNAs were synthesized and messenger RNA (mRNA) expression levels of selected genes were quantified through real-time PCR analysis.

Western blotting

The proteins (40 μg of tissue protein or 15 μg of cell protein) lysed with RIPA buffer (Thermo Scientific, Waltham, MA) were separated using 12 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA), which were then blocked and incubated with an appropriate primary antibody dilution. Protein bands were detected using an enhanced chemiluminescence system Western blot detection kit (Tiangen, Beijing, China) after probing with secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and were visualized by exposure to X-ray film (Kodak, NY, USA). The intensities of protein bands were quantified using Quantity One software (Bio-Rad, Hercules, CA).

TaqMan microRNA (miRNA) assay

About 50 ng of RNA from the liver and Hepa 1-6 cells, respectively, were used for TaqMan miRNA assay, as mentioned in our previous report (Yan et al. 2014).

Statistical analysis

Statistical analyses were performed using SPSS for Windows 17.0 Software (SPSS, Inc., Chicago, IL, USA). Data were represented as means with standard errors (mean ± SE). Differences between treatments were determined using one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test, with p values of <0.05 deemed significant. All represented mean ± SE from cell experiments were based on at least three independent experiments.

Results

PFOA changed lipid content and activated PPARs in mouse liver

To investigate the effects of PFOA on hepatic lipid metabolism, mice were exposed to PFOA by gavage for 28 days, and liver TCHO and TG concentrations were then analyzed (Fig. 1a, b). Results demonstrated that TCHO concentrations in the liver were reduced in a dose-dependent manner after exposure to PFOA, and TG concentrations were reduced at doses of 0.08 and 20 mg/kg/day but increased at a dose of 1.25 mg/kg/day. The concentrations of PFOA were about 0.26 μg/g in the livers of mice dosed with Milli-Q water and increased in a dose-dependent manner after 28-day exposure (Fig. 1c).

The mRNA levels of PPARs and its representative target genes were detected using real-time PCR analysis in livers of mice exposed to PFOA (Fig. 1d, e). Results showed slight changes in PPARα mRNA expression at low doses of PFOA, while PPARγ mRNA expression was significantly increased in a dose-dependent manner; however, PPARβ/δ mRNA expression exhibited no differences among the groups. The mRNA levels of most representative PPARα target genes selected for this study were altered in the livers of mice exposed to PFOA at doses of 1.25 mg/kg/day and higher, except for Apoa4, which increased at doses of 0.08 and 20 mg/kg/day.

PFOA increased mRNA expression of SREBP target genes, and SREBPs maturation was robustly induced in mouse liver

As mentioned above, although PPARα agonist seems to be the key event during PFOA-induced rodent liver toxicity, studies on PFOA-exposed PPARα-null mice have indicated that other mechanisms for effects induced by PFOA exposure on livers are involved. Accordingly, transcription factors SREBP and HNF4α, as well as their representative target genes, were analyzed at the transcriptional level in livers of PFOA-exposed mice to determine other possible mechanisms for liver toxicity induced by PFOA exposure (Fig. 2a, b, Fig. S3). No differences were observed in the representative HNF4α target genes, although HNF4α was reduced at the transcriptional level in livers of mice dosed with 1.25 and 5 mg/kg/day PFOA compared with the control. Transcriptional products of SREBP genes were also examined, and the expression of SREBP1 mRNA was

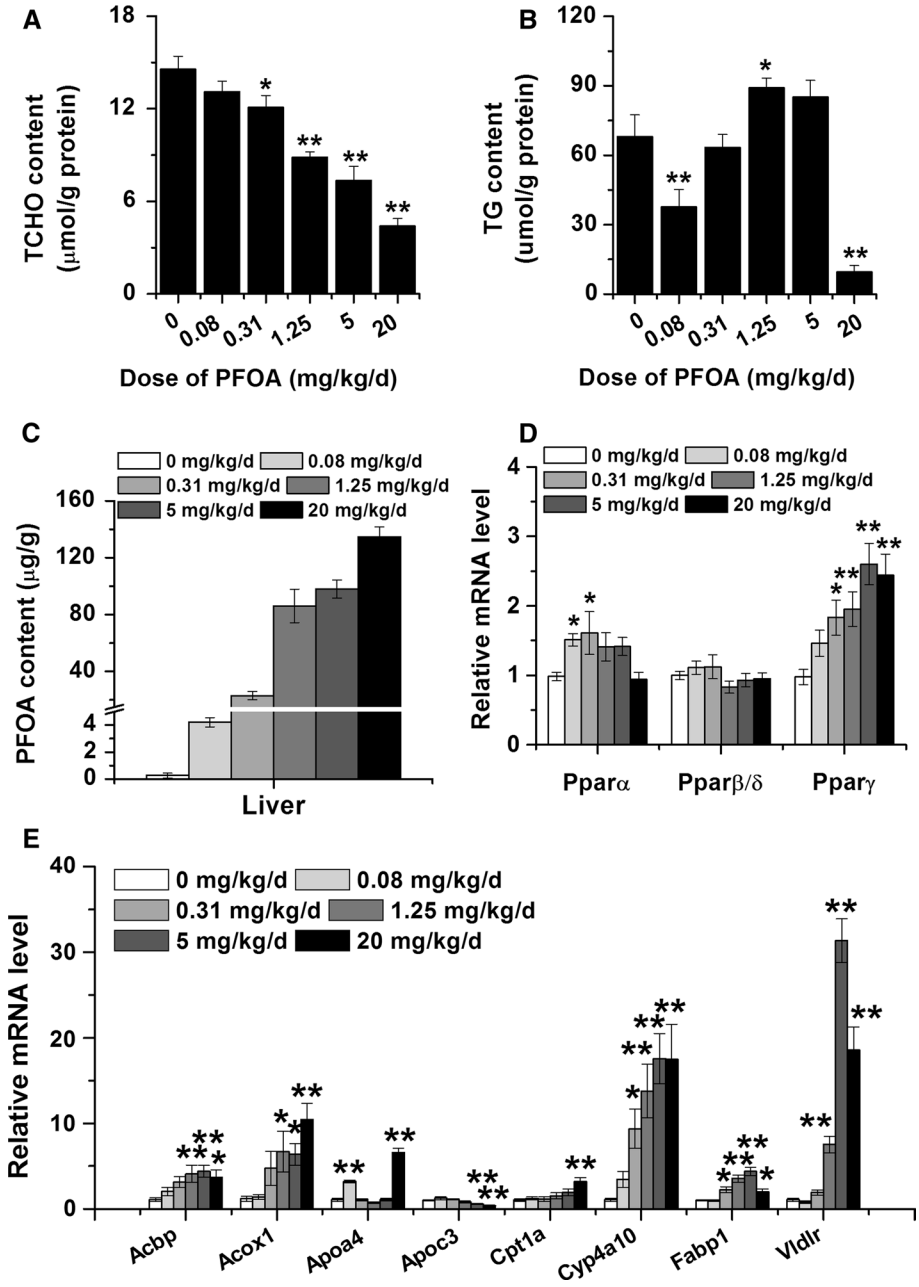

Fig. 1 Liver lipid content and relative mRNA level of PPAR-related genes in livers of mice after 28-day exposure to PFOA. **a** TCHO contents and **b** TG contents expressed per gram liver ($n = 8$). **c** PFOA contents expressed per gram liver ($n = 3$). **d** Relative expression of PPARs mRNAs ($n = 6$). **e** Relative mRNA level of representative PPAR-α target genes ($n = 6$). Data are mean ± SE. Significantly different from control group (*$p < 0.05$; **$p < 0.01$)

induced from doses of 0.31 to 5 mg/kg/day and expression of SREBP2 mRNA was slightly induced at the dose of 20 mg/kg/day. The mRNA levels of representative SREBP target genes selected for this study were significantly increased. Our results demonstrated that SREBPs were activated in livers of mice after exposure to PFOA for 28 days. Because the activation of SREBPs was directed by the cleavage of SREBPs precursors, we detected protein expression of SREBPs by Western blotting, and the results indicated that the precursor of SREBP1 and SREBP2 reduced significantly and the cleavage fragments of them increased significantly (Fig. 2c, d).

Protein expressions of key regulators in the SREBP maturation pathway changed in the mouse liver after exposure to PFOA

Due to the activation of SREBPs, we observed changes in the SREBP maturation pathway in livers of mice after 28-day exposure to PFOA. Key factors involved in SREBP maturation were analyzed at both the transcriptional and protein levels (Fig. S4, Fig.3a, b). Interestingly, although no significant changes were found at the transcriptional level, the protein levels of these factors, including INSIG1 and INSIG2 (proteins blocking ER-to-Golgi transport of

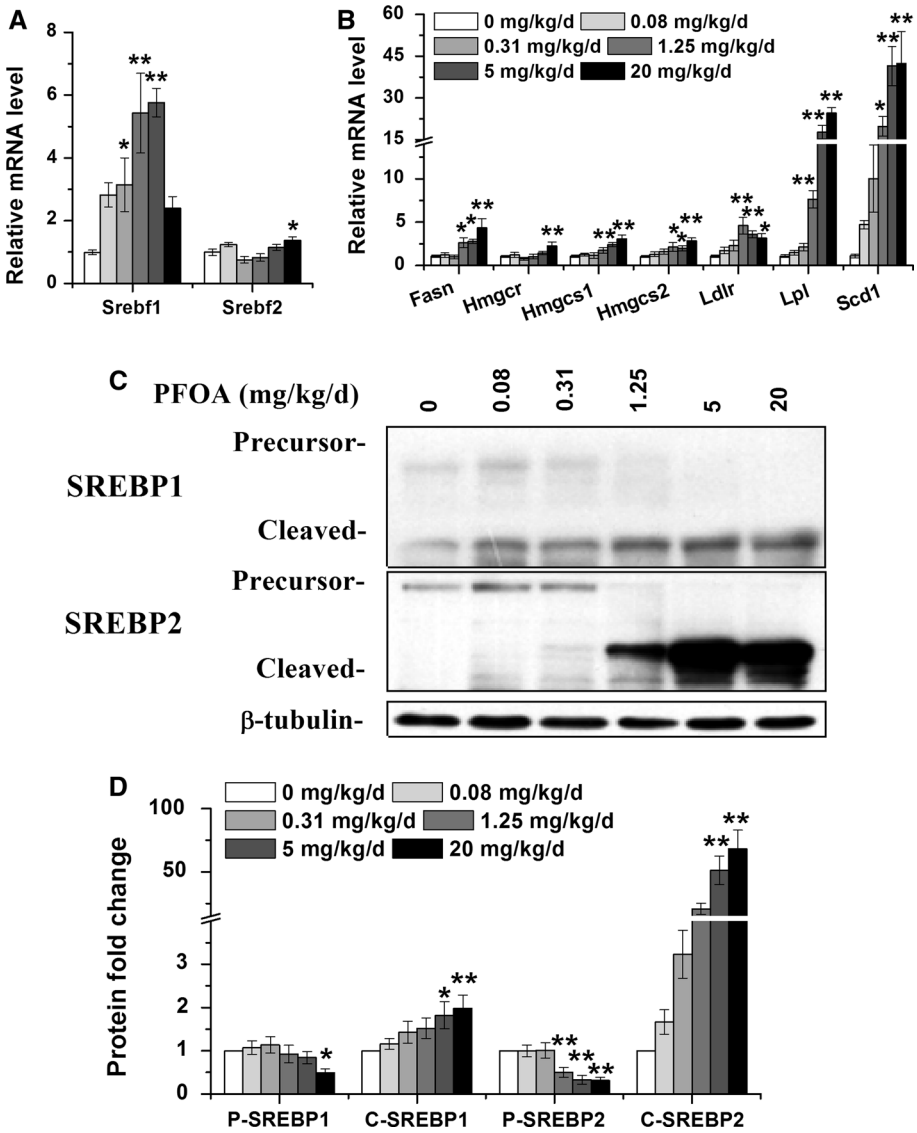

Fig. 2 Relative mRNA level of SREBP-related genes and SREBP protein levels in livers of mice after 28-day exposure to PFOA. **a** Relative mRNA level of SREBPs ($n = 6$). **b** Relative mRNA level of representative SREBP target genes ($n = 6$). **c** SREBP protein expression and **d** relative fold change of band densities ($n = 3$). Data are mean ± SE. Significantly different from control group (*$p < 0.05$; **$p < 0.01$)

SREBP), F-box and WD-40 domain protein 7 (FBXW7, protein-mediated nucleus SREBP ubiquitinylation and proteasomal degradation), and SREBP cleavage-activating protein (SCAP, protein-mediated transport of SREBP precursor), all decreased significantly. These results demonstrated that proteins involved in blocking SREBPs transport and nuclear SREBPs degradation all decreased in livers of mice after 28-day exposure to PFOA, indicating the activation of the SREBP maturation pathway.

Earlier research determined that a SREBP-controlling miRNA cluster, miR-183-96-182, was involved in a regulatory loop intermediated by SREBP maturation (Jeon et al. 2013). Using TaqMan miRNA assay, we also found a significant increase in the expression level of the miR-183-96-182 cluster (Fig. 3c) in the livers of mice after 28-day exposure to PFOA, indicating the possible involvement of miRNAs during PFOA-induced effects on the liver.

PFOA activated PPARs but did not affect TCHO and TG concentrations in Hepa 1-6 cells

To further explore the effects of PFOA on SREBP maturation and lipid metabolism, especially the potential correlation between SREBP activation and PPAR, mouse hepatocellular carcinoma cell line Hepa 1-6 was used for in vitro study. Lipid content of cells was detected using fluorescent neutral lipid dye BODIPY 493/503 by flow cytometry. Results showed that lipid content in Hepa 1-6 cells significantly increased after 72-h exposure to PFOA (Fig. 4a). Concentrations of TCHO and TG in Hepa 1-6 cells after exposure to PFOA for 72 h exhibited no significant change (Fig. 4b, c). It would seem, therefore, the increase in the lipid content induced by PFOA exposure was not attributable to TCHO and TG accumulation in Hepa 1-6 cells.

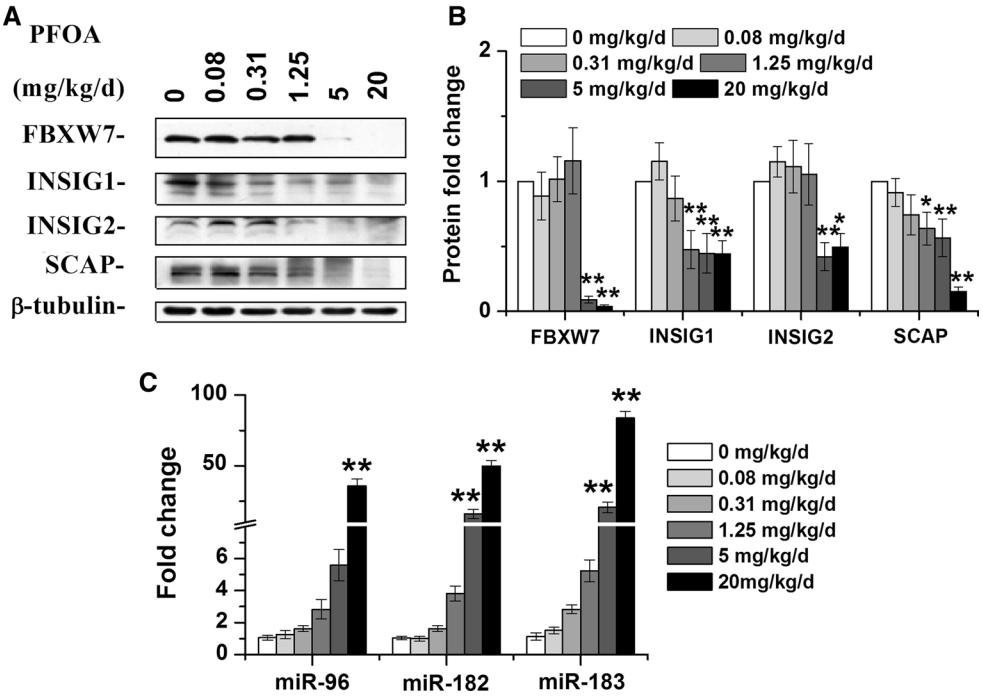

Fig. 3 Protein expression of key factors in the SREBP maturation pathway and miR-183-96-182 cluster expression in livers of mice after 28-day exposure to PFOA. **a** FBXW7, INSIG1, INSIG2, and SCAP protein expression and **b** relative fold change of band densities ($n = 3$). **c** Relative fold change of miR-183-96-182 cluster expression ($n = 6$). Data are mean ± SE. Significantly different from control group (*$p < 0.05$; **$p < 0.01$)

PPARα and PPARγ gene expressions were also analyzed in Hepa 1-6 cells exposed to PFOA for 72 h (Fig. 4d). No significant changes were observed at the transcriptional level, but mRNA levels of the PPARα representative target genes increased significantly, except for Acox1, indicating that PPARα was activated in Hepa 1-6 cells after exposure to PFOA. To explore the relationship between PPARα and SREBP activation, a PPARα ligand (WY-14,643) was used as positive control. As shown in Fig. 4d, only Cpt1a mRNA expression was significantly induced in Hepa 1-6 cells after 72-h exposure to WY-14,643. However, in the human hepatoma cell line HepG2, the expressions of Cpt1a and Fabp1 mRNAs were significantly induced at the same dose of WY-14,643 for the same exposure time (data not shown), suggesting that PPARα in Hepa 1-6 cells was not sensitive to the PPARα ligand WY-14,643 but was sensitive to PFOA.

SREBP maturation pathway was not induced in Hepa 1-6 cells after PFOA exposure

Based on the pattern of lipid content and PPAR-related gene expression, we explored the effects of PFOA on SREBP activation in Hepa 1-6 cells. Expression of the SREBP1 and SREBP2 genes was reduced at the transcriptional level in Hepa 1-6 cells after exposure to PFOA, but the expression in two representative SREBP target genes, fatty acid synthase (Fasn) and 3-hydroxy-3-methylglutaryl coenzyme A reductase (Hmgcr), did not change (Fig. 5a). The mRNA levels of SREBP and their representative target genes did not change after exposure to WY-14,643 (Fig. 5a). We also analyzed the miR-183-96-182 cluster using TaqMan miRNA assay, but only miR-182-5p was detected in Hepa 1-6 cells and its expression did not change after PFOA exposure (Fig. 5b).

Protein levels of SREBP1 and SREBP2 were also analyzed using Western blotting (Fig. 5c, d), which demonstrated that SREBP cleavage was not activated after exposure to PFOA or WY-14,643. Proteins involved in the SREBP maturation pathway, namely, FBXW7, INSIG1, INSIG2, and SCAP, were also detected without significant change after exposure to PFOA or WY-14,643 (Fig. 5c, d). These results indicated that SREBP maturation was not stimulated by PFOA in Hepa 1-6 cells.

Discussion

Studies in rodents have demonstrated that PFOA and PFOS can initiate sequential morphological and biochemical events in the liver, like hepatocellular hypertrophy due to peroxisome proliferation, fatty acid oxidation, and many alterations in lipid metabolism, and PPARα activation has been suggested as the key factor (Kennedy et al. 2004; Lau et al. 2007). Earlier studies reported that PFOA can interfere with both fatty acid and cholesterol synthesis in the rat liver, resulting to a reduction in TCHO and TG serum levels but increase in the liver (Haughom and Spydevold 1992; Kennedy et al. 2004; Kudo et al. 1999). Previous research showed an increase in TG concentration and no

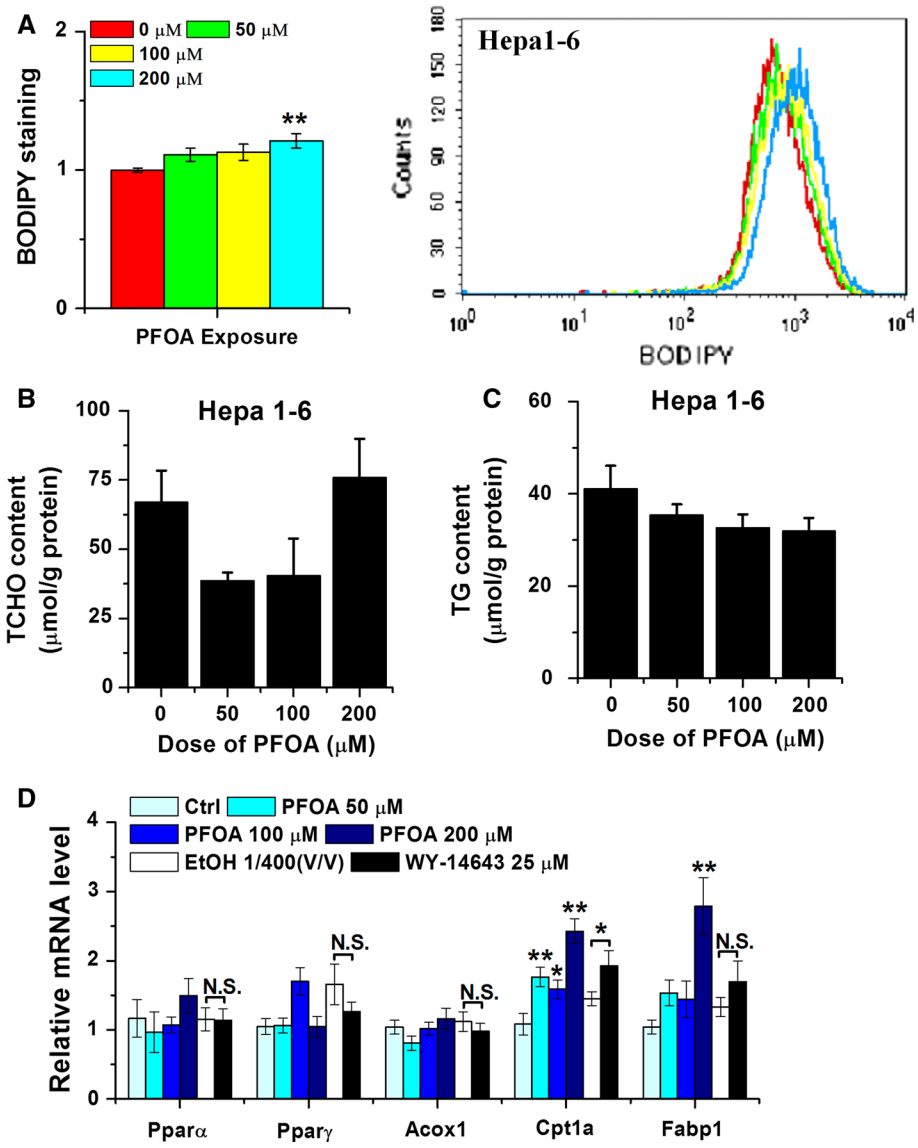

Fig. 4 Lipid content and mRNA expression of PPAR-related genes in Hepa 1-6 cells after 72-h exposure to PFOA. **a** Fluorescence intensity fold change of BODIPY 493/503 staining and representative flow cytometry histogram data. **b** TCHO and **c** TG contents expressed per gram cell protein. **d** Relative fold change of PPARs and PPAR-α representative target genes mRNA level. Data are mean ± SE from each of the three experiments was performed in triplicate. Significantly different from control group or ethanol group (*$p < 0.05$; **$p < 0.01$)

significant change in TCHO and free fatty acid concentrations in mouse liver following 21-day exposure to PFOA at 5 mg/kg/day, but decreased concentrations of free fatty acid in serum (Tan et al. 2013). In our present study, although PPARα was activated in mouse liver after 28-day exposure to PFOA, TCHO content was reduced in a dose-dependent manner and TG content was also reduced at the lowest (0.08 mg/kg/day) and highest (20 mg/kg/day) doses, which was in agreement with the patterns found in previously analyzed serum (Yan et al. 2014). These discrepancies between our results and those of earlier reports may originate from the different exposure doses and times or species differences between rats and mice and require further study.

While PPARα activation seems like a key event during PFOA-induced liver toxicity and may contribute to the disturbance of lipid metabolism, previous study has also revealed PPARα-independent effects in PPARα-null mice exposed to PFOS or PFOA for 7 days (Rosen et al. 2010). In addition, several reports have demonstrated that, unlike normal PPARα agonists, PFOA exposure increased hepatocyte hypertrophy and altered the liver gene expression profile in PPARα-null mice (Minata et al. 2010; Rosen et al. 2008a, b; Wolf et al. 2008). An in vitro study on the activation of multiple nuclear receptors, including PPARα, pregnane X receptor (PXR), constitutive active receptor (CAR), LXR-α, and farnesoid X receptor (FXR), in the primary hepatocytes of rats and humans exposed to PFOA or PFOS (Bjork et al. 2011) demonstrated that PFOA and PFOS could activate nuclear receptors other than PPARα. To explore whether transcription factors other than PPARα were activated after exposure to PFOA in mice, we analyzed the expression of genes related to HNF4α and SREBPs at the transcriptional level. Earlier research suggested that HNF4α expression decreased after exposure

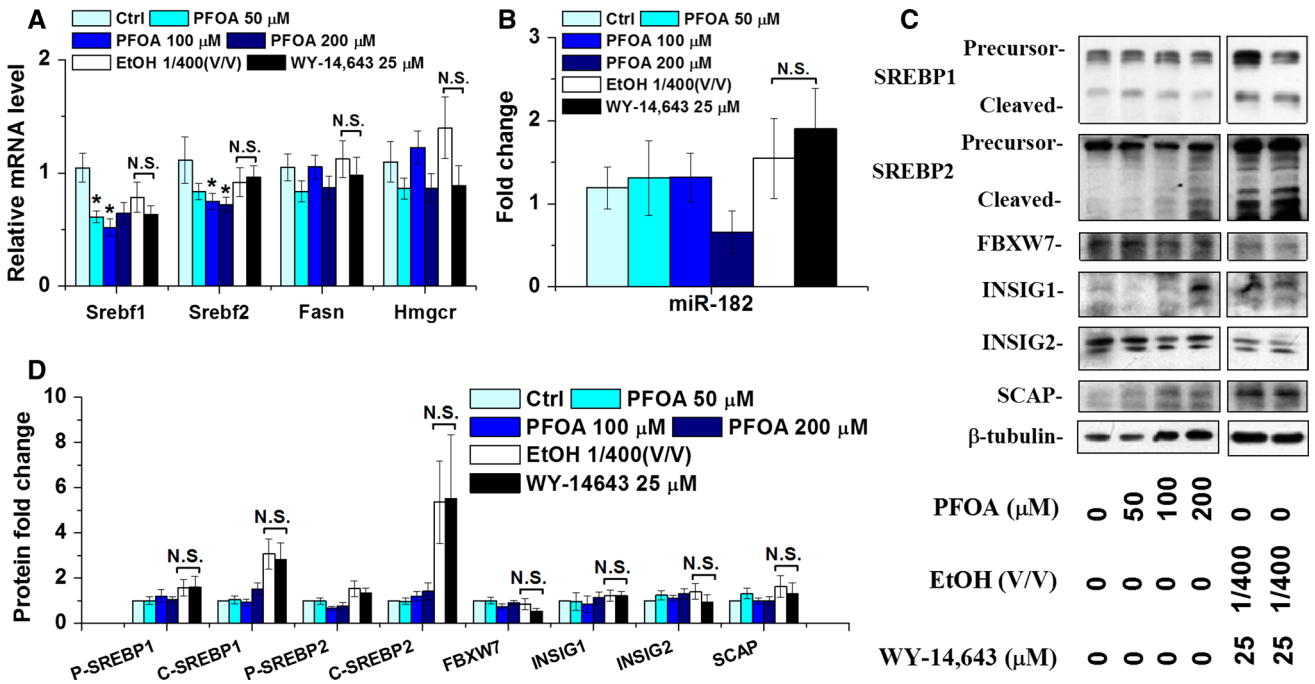

Fig. 5 Relative mRNA level of SREBP-related genes and alteration in the SREBP maturation pathway in Hepa 1-6 cells after 72-h exposure to PFOA. **a** Relative fold change of SREBPs and their representative target genes mRNA level, each of the three experiments was performed in triplicate. **b** Relative fold change of miR-182 expression, three independent experiments performed in triplicate. **c** SREBPs and key factors in maturation proteins expression and **d** relative fold change of bands density, three independent experiments performed in duplicate. Data are mean ± SE. Significantly different from control group or ethanol group (*$p < 0.05$; **$p < 0.01$)

to PFOA in vitro (Scharmach et al. 2012). In the present study, the HNF4α mRNA level was slightly declined in mouse liver after PFOA exposure; however, there were no significant changes in representative HNF4α target genes at the transcriptional level. The induction of SREBP1c gene expression at the transcriptional level by PFAAs has been reported (Bjork et al. 2011; Fang et al. 2012) but without further investigation. Our study demonstrated that PFOA exposure can increase mRNA levels of SREBP1 as well as its target genes in mouse liver, which suggests the activation of SREBP following PFOA exposure.

The transcriptional regulation of SREBPs is different in different tissues, and the transcription of SREBPs is generally enhanced by SREBPs through a feed-forward mechanism, LXR-retinoid X receptor (RXR) heterodimers, and insulin signaling (Bengoechea-Alonso and Ericsson 2007; Jeon and Osborne 2012; Raghow et al. 2008). Several studies have investigated the cross talk between PPAR and SREBP using PPAR- or SREBP-null mice and PPAR agonists. Alteration of SREBP gene expression can be induced by representative PPARα agonists at the transcriptional level (Gao et al. 2013). Knight et al. (2005) determined that the PPARα ligand, WY-14,643, induced SREBP1 activation by enhancing precursor cleavage but not its mRNA expression, and these changes were not apparent in PPARα-null mice in the particular experiment. Our present data also showed the induction of SREBP1 and 2 precursor cleavage, together with an increase in SREBP1 mRNA levels the same time, in the livers of mice after 28-day exposure to PFOA. These findings indicated that there were different effects in mouse liver exposed to PFOA or WY-14,643. From earlier in vitro studies, SREBP1 gene expression was enhanced by PPARα agonists, but inhibited by overexpression of PPARα and γ (Fernandez-Alvarez et al. 2011; Yoshikawa et al. 2003). Our in vitro data from PFOA-exposed Hepa 1-6 cells demonstrated that PFOA could activate PPARα function and reduce SREBP1 and 2 mRNA levels, but there were no effects on SREBP transcriptional activity. Furthermore, Hepa 1-6 cells exposed to WY-14,643 exhibited PPARα activation but no effects on SREBPs. Unlike previous studies, our results indicated that Hepa 1-6 cells were sensitive to PFOA but not WY-14,643, and PPARα activation resulted in a reduction in SREBP mRNA levels without SREBP activation.

SREBPs exist as transcriptionally inactive membrane-bound precursor proteins after their biosynthesis on rough endoplasmic reticulum (ER) membranes. They are released as soluble and nuclear-target SREBPs through an elaborate ER-to-Golgi transport and proteolytic activation (Jeon and Osborne 2012). During SREBP maturation, SREBP precursors form a complex with SCAP in the ER membranes and are then transported to the Golgi via COPII vesicles

where membrane-bound transcription factor peptidase, site 1 (S1P) and S2P sequentially cleave the SREBP precursor and release the mature soluble SREBP transcription factor from the membrane to the nucleus (Jeon et al. 2013; Shao and Espenshade 2012). SREBP–SCAP complex transport is blocked by interaction with ER-resident INSIGs, which are encoded by two insulin-induced genes, Insig1 and Insig2 (Jeon et al. 2013; Shao and Espenshade 2012). We detected SREBPs and four important regulation factors during SREBP maturation, with all protein expression levels decreasing in the mouse liver after PFOA exposure, indicating that the SREBP maturation pathway was activated in the mouse liver following treatment with PFOA. Konig et al. (2009) found that activation of PPARα and PPARγ using WY-14,643 and troglitazone reduced nuclear SREBP1 by increasing INSIGs in rat hepatoma cells. Unlike earlier reports using representative PPAR agonists, we did not detect increased INSIGs or decreased cleaved SREBP proteins in Hepa 1-6 cells exposed to PFOA or WY-14,643. As a sterol-regulated escort protein, SCAP is important for SREBP maturation and lipid synthesis (Matsuda et al. 2001; Osborne 2001). A reduction in SCAP protein expression was observed in the mouse liver after PFOA exposure in this study; however, SREBP activation was still stimulated. The reason for these findings need further study.

miRNAs are typically ~23 nucleotides long, single-strand noncoding RNAs capable of directing protein-coding gene posttranscriptional repression by pairing to mRNAs (Bartel 2009). Multiple studies have demonstrated that miRNAs are regulated under metabolic stimuli and conversely control metabolism (Dumortier et al. 2013). The miRNA cluster, miR-183-96-182, has been reported to contribute to a regulatory loop intermediated by SREBP maturation for intracellular lipid homeostasis and is also thought to be regulated by SREBPs (Jeon et al. 2013). Here, we found that miR-183-96-182 cluster levels were significantly enhanced in mouse liver after treatment with PFOA. Both FBXW7 and INSIG2, which are suppressed by miR-182 and miR-96, respectively, were also significantly suppressed in the mouse liver after PFOA exposure. These results indicated that miRNAs were involved in perturbing liver lipid metabolism after PFOA exposure. However, in vitro results from PFOA-treated Hepa 1-6 cells only detected miR-182, and its expression level showed no significant change, which needs further exploration.

As mentioned above, SREBP activation by stimulation of the SREBP maturation pathway in mouse liver exposed to PFOA in vivo was not found in Hepa 1-6 cells after PFOA treatment in vitro. SREBPs are suggested to be the center of the feedback system monitoring changes in cholesterol levels and regulating lipid metabolism (Bengoechea-Alonso and Ericsson 2007). PFOA is suggested as a PPARα ligand and is capable of activating PPARα. Although PPARα and SREBPs play important roles in lipid metabolism, PPARα mostly regulates fatty acid oxidation systems to increase energy combustion (Pyper et al. 2010), while SREBPs regulate cellular cholesterol and fatty acid homoeostasis in the liver (Horton 2002; Horton et al. 2002). Hypolipidemic effects due to PPARα and PFOA exposure in rodents are significant (Kennedy et al. 2004; Pyper et al. 2010). Our previous study found the reduction of serum TCHO (Yan et al. 2014), and we also found a reduction in liver TCHO in the present study. This observation in the liver of mice exposed to PFOA may be explained by the reduction in serum TCHO due to PPARα activation resulting in cholesterol deficiency in the liver, followed by SREBP maturation. Whether this phenomenon is a compensatory response elicited from hypolipidemic effects induced by PFOA exposure still needs further investigation. A possible schematic model of how the SREBP maturation pathway is involved in the effects induced by PFOA exposure on liver metabolism is shown in Fig. S5.

Our research indicated that SREBPs were activated by PFOA exposure in mouse liver through stimulation of the SREBP maturation pathway. The decrease in INSIGs and FBXW7 and induction of miR-183-96-182 cluster expression also appears to be involved in this event. The SREBP pathway may play an important role in PFOA-induced lipid metabolism turbulence in the liver.

Acknowledgments This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB14040202) and the National Natural Science Foundation of China (Grant 31320103915, 31025006 and 21277143).

Conflict of interest The authors declare there are no conflicts of interest.

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