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Fat-specific protein 27 is a novel target gene of liver X receptor α

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ABSTRACT

Fat-specific protein 27 (FSP27) is highly expressed in the fatty liver of genetically obese *ob/ob* mice and promotes hepatic triglyceride (TG) accumulation. The nuclear hormone receptor liver X receptor α (LXRα) also plays a critical role in the control of TG levels in the liver. The present study demonstrated transcriptional regulation of *Fsp27a* and *Fsp27b* genes by LXRα. Treatment with the LXR ligand T0901317 markedly increased *Fsp27a* and *Fsp27b* mRNAs in wild-type C57BL/6J and *ob/ob* mouse livers. A reporter assay indicated that two LXR-responsive elements (LXREs) are necessary for LXRα-dependent induction of *Fsp27a* and *Fsp27b* promoter activities. Furthermore, the LXRα/retinoid X receptor α complex is capable of directly binding to the two LXREs both *in vitro* and *in vivo*. These results suggest that LXRα positively regulates *Fsp27a* and *Fsp27b* expression through two functional LXREs. *Fsp27a/b* are novel LXR target genes in the *ob/ob* fatty liver.

1. Introduction

Fat-specific protein 27 (FSP27) was initially isolated by a differential screening approach using mouse adipocyte TA cell lines expressing a mature adipocyte-specific gene (Williams et al., 1992; Danesch et al., 1992). FSP27 belongs to the cell death-inducing DNA fragmentation factor 45-like effector (CIDE) family, which consists of three proteins: CIDEA, CIDEB, and FSP27. The human homolog of mouse FSP27 was also reported as CIDEC (Liang et al., 2003). FSP27 was found to be highly expressed in white and brown adipose tissue and localized to lipid droplets (LDs) in adipocytes (Puri et al., 2007). FSP27 promotes the formation of LD–LD fusions on adipocytes (Gong et al., 2011; Jambunathan et al., 2011) and enlarged unilocular LDs in cooperation with perilipin 1, another LD-associated protein (Sun et al., 2013). *Fsp27*-null mice showed protection from diet-induced obesity and insulin resistance, and exhibited a small mass of white adipose tissue and the presence of multilocular LDs (Toh et al., 2008; Nishino et al., 2008). Adipocyte-specific *Fsp27*-null mice also exhibited small white adipose tissue masses and hepatic steatosis (Tanaka et al., 2015).

Previous studies demonstrated that hepatic peroxisome proliferator-activated receptor γ (PPARγ) promoted triglyceride (TG) accumulation and fatty liver development in *ob/ob* mice, a well-characterized model of type 2 diabetes, obesity, and fatty liver because of its mutated *leptin* gene (Matsusue et al., 2003). Furthermore, FSP27 was established as the direct mediator of PPARγ-dependent hepatic steatosis in *ob/ob* mice. Interestingly, FSP27 expression showed the highest levels in *ob/ob* fatty liver, but lower levels in normal liver and led to an increase in hepatic TG levels (Matsusue et al., 2008). Recently, FSP27 isoforms were identified as FSP27α and FSP27β. FSP27β contains an additional 10 amino acids at the N-terminus of the original FSP27 identified in *ob/ob* fatty liver (named as FSP27α) and shows higher intracellular stability than FSP27α. It was also demonstrated that both isoforms directly promote hepatic TG accumulation (Xu et al., 2015).

Liver X receptor (LXR) α and β are members of a family of ligand-dependent nuclear receptors (Mangelsdorf et al., 1995). LXRs heterodimerize with retinoid X receptors (RXR) and regulate

Abbreviations: FSP27, fat-specific protein 27; CIDE, cell death-inducing DNA fragmentation factor 45-like effector; LD, lipid droplet; TG, triglyceride; PPAR, peroxisome proliferator-activated receptor; CREBH, cyclic AMP-responsive element binding protein H; LXR, liver X receptor; LXRE, LXR response element; FAS, fatty acid synthase; SCD1, stearoyl-coenzyme A desaturase 1; PFK2, phosphofructokinase-2; SREBP1c, sterol regulatory element-binding protein-1c; ChREBP, carbohydrate response element-binding protein; ACC, acetyl CoA carboxylase; GPAT, glycerol-3-phosphate acyltransferase; UTR, untranslated region; ob/ob, leptin-deficient mice; OB/OB, C57BL/6J wild-type for leptin gene.

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transcription by binding to LXR-responsive elements (LXRE) of target genes. LXRs play important roles in regulating genes associated with lipogenesis in the liver (Baranowski, 2008). Indeed, activation of LXRs by the LXR ligand, T0901317, caused a marked increase in hepatic TG levels and aggravation of fatty liver in *ob/ob* or other fatty liver model mice (Matsusue et al., 2014; Chisholm and Chisholm, 2003). Regarding hepatic fat accumulation, two transcription factors, sterol regulatory element-binding protein 1c (SREBP1c) and carbohydrate response element-binding protein (ChREBP), play crucial roles in LXR-mediated hepatic lipogenesis (Baranowski, 2008; Matsusue et al., 2014). SREBP1c and ChREBP are direct targets of LXRs and control the expression of nearly all genes integral to lipogenesis, including fatty acid synthase (*Fas*), acetyl-CoA carboxylase, and stearoyl-CoA desaturase 1 (*Scd1*) (Baranowski, 2008; Matsusue et al., 2014). Thus, LXR signaling mediated by SREBP1c and ChREBP is thought to contribute to an increase in hepatic TG content by upregulating these lipogenic genes. Whether LXRs induce not only lipogenic genes but also *Fsp27a/b* in *ob/ob* fatty liver is unknown. Additionally, the transcriptional regulation of hepatic *Fsp27a/b* by LXRs remains unclear.

In the present study, administration of T0901317 to C57BL/6J mice wild-type for the *leptin* gene (*OB/OB*) and *ob/ob* mice showed markedly increased *Fsp27a* and *Fsp27b* expression. Furthermore, two functional LXREs were identified within 5′-upstream regions of *Fsp27a* and *Fsp27b*. These findings suggest that *Fsp27a* and *Fsp27b* are directly regulated by LXRα and a novel LXRα target gene in the liver.

2. Materials and methods

2.1. Animals and treatment

All animal protocols and studies were performed according to guidelines from the Center for Experimental Animals at Fukuoka University. Eight-week-old male mice (n = 4) on an *ob/ob* background or C57BL/6J mice with a wild-type *leptin* gene (*OB/OB*) were fed an *ad libitum* diet (MF, Oriental Yeast, Fukuoka, Japan) with or without 0.025% (w/w) T0901317 (Sigma Aldrich, St. Louis, MO, USA) for 2 weeks, as previously described (Matsusue et al., 2014). GW3965 (Selleck, Japan) was administered with 20 mg/day/kg for 3 days by oral gavage (Laffitte et al., 2003). As a positive control for oral gavage administration, T0901317 was administered at 20 mg/day/kg for 3 days (Jakel et al., 2004). Vehicle alone was administered as a negative control (0.5% methyl-cellulose).

2.2. Total RNA isolation and qPCR

RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA), and quantitative polymerase chain reaction (qPCR) was performed using cDNA generated from 1 μg of total RNA with an AffinityScript qPCR cDNA Synthesis kit (Agilent Technologies, Santa Clara, CA, USA). The primer sequences used were described previously for the following genes: *Fsp27a* and *Fsp27b*, (Xu et al., 2015); *Lxra*, *Srebp1c*, *Fas*, *Scd1*, *Gpat*, and *Pparg* (Matsusue et al., 2014).

2.3. Cell culture

HEK293FT cells were cultured at 37 °C under 5% CO_2 in Dulbecco's Modified Eagle's Medium with high glucose and pyruvate (Thermo Fisher Scientific), supplemented with 10% fetal bovine serum (Biowest, Nuaillé, France) and 1% Antibiotic-Antimycotic (Thermo Fisher Scientific).

2.4. Construction of reporter and expression plasmids

The transcriptional start sites of mouse *Fsp27a* and *Fsp27b* were determined previously (Danesch et al., 1992; Xu et al., 2015). The −1698 (A1), −1426 (A2), −1267 (A3), and −139 (A4) base pair (bp) fragments from the transcriptional start site (+1) of the mouse *Fsp27a* 5′-upstream region, containing KpnI and MluI sites at the 5′- and 3′-end of the primers, were amplified by PCR and cloned into the luciferase reporter vector pGL3 basic (Promega, Madison, WI, USA) as previously described (Matsusue et al., 2008).

Internal deletion constructs for *Fsp27a* were prepared by inverse PCR. The *Fsp27a* A4 construct was used as a template. The primer sequences were as follows: A4-1forward, 5′-GGAGCTGGGGTATATGGC-3′ and reverse, 5′-CCCAGCCTCCTGGCAATA-3′; A4-2 forward, 5′-ATGGCTGAGGTCGCAGTT-3′ and reverse, 5′-CCATGTCCCTTATATACC-3′; A4-3 forward, 5′-ATAAGGGACATGGTTGGA-3′ and reverse, 5′-ATATACCCCAGCTCCTCA-3′.

The −2647 (B1), −2375 (B2), −1382 (B3), and −806 (B4) bp fragments from the transcriptional start site (+1) of the mouse *Fsp27b* 5′-upstream region, containing CACC sites at the 5′-end of the primers, were amplified by PCR and cloned into the Gateway entry vector pENTR/D-TOPO (Thermo Fisher Scientific), and then recombined into the destination vector pGL4.17 (Promega), which was prepared using the Gateway Vector Conversion System according to the manufacturer's instructions (Thermo Fisher Scientific). The primer sequences were as follows: B1 forward, 5′-CACCCTCCCATTGCTCATTCG-3′; B2 forward, 5′-CACCATCAGCTGTGCCTACGGATG-3′; B3 forward, 5′-CACCTGAGACAGGGCCAACTCT-3′; B4 forward, 5′-CACCAGTGTTGGGTTGTGGTGAGG-3′; reverse for all constructs, 5′-TGTTTCTCCGACCCAAGCTG-3′.

The LXRα and RXRα expression vectors were prepared as described previously (Matsusue et al., 2006). The complete open reading frame of mouse LXRβ was amplified by PCR from a mouse liver cDNA library by using gene-specific primers and cloned into the Gateway entry vector, pENTR/D-TOPO (Thermo Fisher Scientific). This sequence was then recombined into the destination vector, pcDNA3.1 (Thermo Fisher Scientific), which was prepared using the Gateway Vector Conversion System (Thermo Fisher Scientific). The primer sequences were as follows: forward, 5′-CACCACCATGGCTTCCCCCACAAGTTCTCTGG-3′ and reverse, 5′-CATCTTCAAGAAGACACCACCAAG-3′.

2.5. Transient transfection and reporter assay

HEK293FT cells were seeded at a density of 2.0×10^5 cells/well in 24-well plates at 24 h prior to transfection. Cells were transfected with plasmids using jetPEI DNA transfection reagent (Polyplus-transfection, Illkirch, France) according to the manufacturer's instructions. Typically, each well contained 2 μL of jetPEI DNA transfection reagent, 0.15 μg of LXRα (or LXRβ) and RXRα expression plasmids, 0.4 μg of luciferase reporter constructs containing the 5′-region of mouse *Fsp27a*/*b*, and 0.05 μg of phRL/TK (Promega) as an internal control for transfection efficiency. After adding the reagents, cells were transfected for 6 h at 37 °C in an atmosphere of 5% CO_2. The cells were then incubated for 42 h in fresh medium containing 10 μM T0901317, 10 μM GW3965, 0.1 μM LG100268 (Sigma-Aldrich), or DMSO. The luciferase assay was performed using the Dual-Luciferase Reporter Assay System (Promega). Luciferase activity was measured using a GENE LIGHT 55A luminometer (Nition, Microtec Co., Ltd., Chiba, Japan).

2.6. In vitro transcription/translation and EMSA

Mouse LXRα and human RXRα proteins were synthesized *in vitro* from LXRα and RXRα expression plasmids using the TNT

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