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Phosphorylation of the liver X receptors

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Abstract The liver X receptors (LXRs) function as nutritional sensors for cholesterol and have important roles in lipid metabolism, glucose homeostasis, and inflammation. We provide the first evidence that LXRs are phosphorylated proteins. Mutational analysis and metabolic labeling indicate LXRα is phosphorylated on serine 198 in the hinge region. This is a consensus target for the MAPK family. A phosphorylation-deficient mutant, LXRα S198A, remains nuclear and responds to ligands like the wild-type protein. The biological significance of LXR phosphorylation remains to be elucidated but could provide a novel mechanism for the regulation of LXR signaling pathways and cellular metabolism.

Keywords: Liver X receptor; Cholesterol metabolism; Phosphorylation

1. Introduction

The liver X receptors (LXRα and LXRβ) have emerged as important regulators of cholesterol metabolism and transport [1]. LXRα is primarily expressed in liver, macrophages, adipose tissue, and the intestinal epithelium. LXRβ is ubiquitously expressed. LXRs function as total body sterol sensors, regulating both cholesterol homeostasis and lipogenesis [2]. The physiologic ligands for LXRs are oxysterols and intermediates in the cholesterol biosynthetic pathway [3,4]. In macrophages, LXRs integrate the response to cellular cholesterol loading and promote reverse cholesterol transport to the liver. They direct transcription of several target genes involved in the cholesterol efflux pathway and repress some inflammatory genes in macrophages [5–7]. LXRs have also been shown to regulate several genes involved in hepatic fatty acid synthesis [8,9].

Previous work has linked LXR signaling to the development of metabolic diseases. Chronic administration of a synthetic LXR ligand significantly reduced atherosclerosis in both LDLR −/− and apoE −/− mice [10]. Furthermore, LXRs are essential for proper cholesterol efflux from macrophages as genetic deletion of the receptor increases lesion development in mice [11]. These studies identify LXRs as atheroprotective factors *in vivo* and directly link LXR activity to the pathogenesis of this disease.

Post-translational modifications such as phosphorylation have been shown to be important regulatory mechanisms for a number of nuclear receptors [12–17]. Here, we establish for the first time that LXRs are phosphorylated proteins. Moreover, we show that LXRα is phosphorylated at a single site (S198) in the hinge region of the protein. This serine is a consensus site for mitogen-activated protein kinase (MAPK) phosphorylation. LXR phosphorylation may constitute a novel mechanism for regulation of LXR-dependent metabolic pathways.

2. Materials and methods

2.1. Reagents

Synthetic LXR ligand, T0901317, was provided by Tim Willson (GlaxoSmithKline). Anti-LXRα antibodies were a generous gift of Jae Bum Kim (Seoul National University, Korea). Orthophosphate (^{32}P) for metabolic labeling experiments was from Amersham. All other chemicals, unless otherwise noted, were from Sigma–Aldrich.

2.2. Plasmid constructs

Full-length coding regions of human LXRα or LXRβ were amplified by PCR using gene-specific primers and subcloned into *Bam*HI/*Kpn*I restriction sites of the following mammalian expression vectors: pShuttle (Stratagene), pCMX, and pEGFP. In some cases, the inserts were epitope-tagged with one to three tandem repeats of the FLAG octapeptide (DYKDDDDK) at the N-terminus for identification by immunoblotting. Deletional mutations for the AF1 (ΔAF1) and hinge regions (ΔHinge) of hLXRα were made as previously described [18]. For retroviral expression constructs, inserts were subcloned into *Bam*HI/*Sal*I sites of the pBabe-Puro vector. Site-directed mutagenesis of wild-type hLXRα was carried out using the QuikChange II method (Stratagene). Sequences of all plasmids were verified by direct DNA sequencing.

2.3. Cell culture

HEK-293 and RAW264.7 macrophages were grown in DMEM (Gibco) supplemented with 10% fetal bovine serum (FBS). For ligand treatments, cells were cultured in RPMI or DMEM medium supplemented with either 10% lipoprotein-deficient serum (Intracel) or 1% FBS (low serum) and the appropriate concentration of T0901317. In some experiments, cells were sterol depleted by adding 5 μM simvastatin (Calbiochem) and 100 μM mevalonic acid during the treatment period.

2.4. Transfections and promoter activity assays

For transient transfections, HEK-293 cells were plated at a density of 5×10^4 cells per well on poly-D-lysine coated 48-well plates. After 24 h, cotransfections were performed by either the calcium phosphate method or using the transfection reagent, Lipofectamine 2000 (Invitrogen). The luciferase reporter was under the control of the rat fatty acid

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Abbreviations: LXR, Liver X Receptor; MAPK, mitogen-activated protein kinase; LDLR, low-density lipoprotein receptor; apoE, apolipoprotein E

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synthase (rFAS) promoter. A renilla luciferase reporter served as an internal control for transfection efficiency. After 24–48 h in ligand-containing or control media, the cells were harvested in lysis buffer and luciferase expression was analyzed by the dual-luciferase assay (Promega), according to the manufacturer's protocol.

2.5. Stable cell lines

FLAG-tagged human LXRα, S198A mutant and LXRβ were cloned into the pBabe-Puro expression vector and packaged into retrovirus by transient transfection of Phoenix E cells. HEK-293 cells or RAW 264.7 macrophages were infected at 50% confluence with equal titers of re-

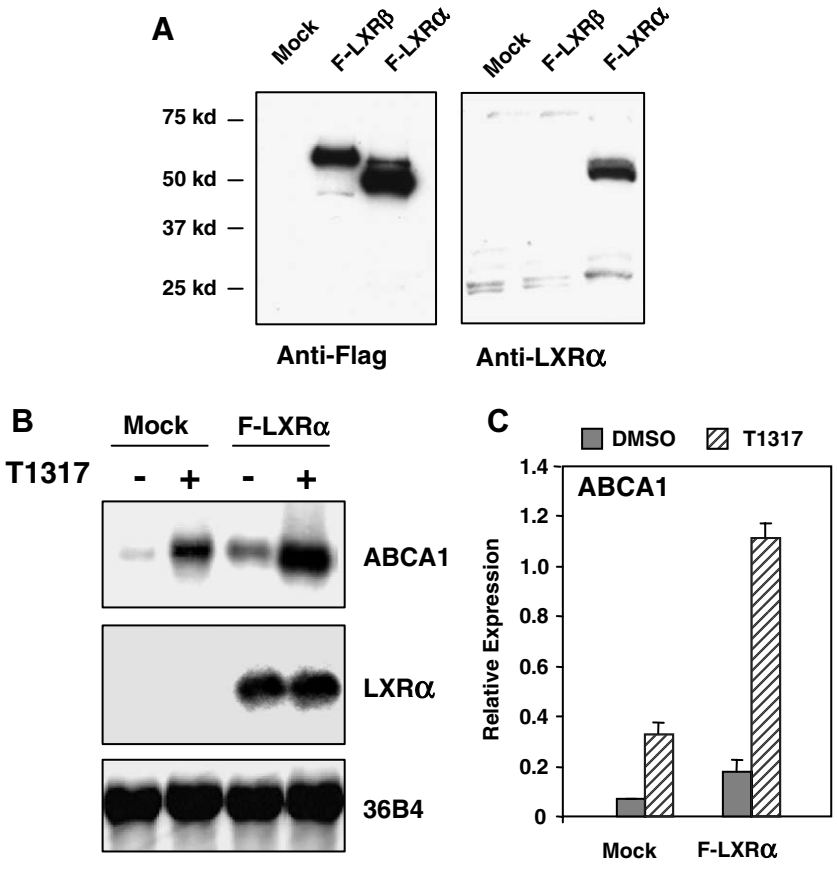

Fig. 1. Generation of stable cell lines. (A) Expression of FLAG-tagged hLXRα or β in stably transduced RAW cells by Western blotting with anti-FLAG and anti-LXRα antibodies. (B) Northern blot analysis of RAW cells stably expressing FLAG-hLXRα. (C) RT-qPCR analysis of ABCA1 expression in RAW cells.

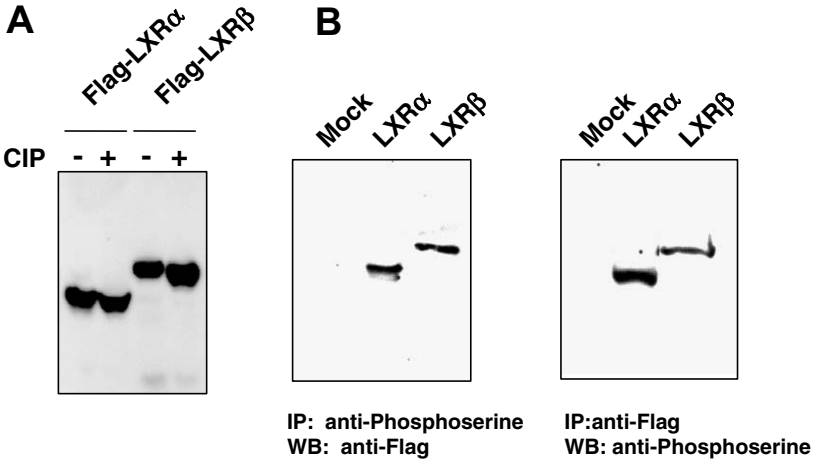

Fig. 2. LXRs are phosphoproteins. (A) Enzymatic dephosphorylation with calf intestinal phosphatase changes the electrophoretic mobility of both LXRα and LXRβ. (B) Co-immunoprecipitation with anti-phosphoserine antibodies confirms that hLXRα and β are phosphorylated.

combinant retrovirus. Stable cell lines were selected with puromycin (2 μg/ml) after 2 weeks of growth. At least two independently derived cell lines were selected to confirm reproducibility of results.

2.6. Quantitative PCR and Northern blot analysis

After lysis in TRIzol Reagent (Life Technologies), total cellular RNA was reverse-transcribed into cDNA according to the manufacturer's protocol using a reverse transcription kit (Roche Molecular Systems). Real-time, quantitative PCR assays for mRNA transcript levels were performed using a Sybergreen-based chemistry as described previously [19]. Samples were analyzed simultaneously for 36B4 expression as the internal control. Quantitative expression values were extrapolated from separate standard curves. Each sample was assayed in duplicate and normalized to 36B4. The sequences for qPCR primers are as follows:

Gene	Primer Direction	qPCR primer sequence
m36B4	Forward 5′ → 3′	AGATGCAGCAGATC-CGCAT
m36B4	Reverse 5′ → 3′	GTTCTTGCCCATCAG-CACC
hLXRα	Forward 5′ → 3′	CTGTGCCTGACATT-CCTCCTG
hLXRα	Reverse 5′ → 3′	CTGGCTGCTTGCAT-CCTGT
mABCA1	Forward 5′ → 3′	GGTTTGGAGATGGTT-ATACAATAGTTGT
mABCA1	Reverse 5′ → 3′	CCCGGAAACGCAAGTCC
mABCG1	Forward 5′ → 3′	TCACCCAGTTCTGCA-TCCTCTT
mABCG1	Reverse 5′ → 3′	GCAGATGTGTCAGGA-CCGAGT

For Northern blots, 10 μg of total cellular RNA was separated by gel electrophoresis, transferred to nylon membranes, and hybridized with [^{32}P]dCTP-labeled DNA probes specific for human 36B4, LXRα, and ABCA1 [19]. Blots were normalized using cDNA probes to 36B4 and quantified by PhosphorImager (Molecular Dynamics) analysis.

2.7. Immunoprecipitation and western blot analysis

FLAG-tagged human LXRα and LXRβ were stably expressed in HEK-293 cells and immunoprecipitated from lysates with anti-FLAG antibody-coated beads (Sigma). Total cellular protein (50–100 μg) was

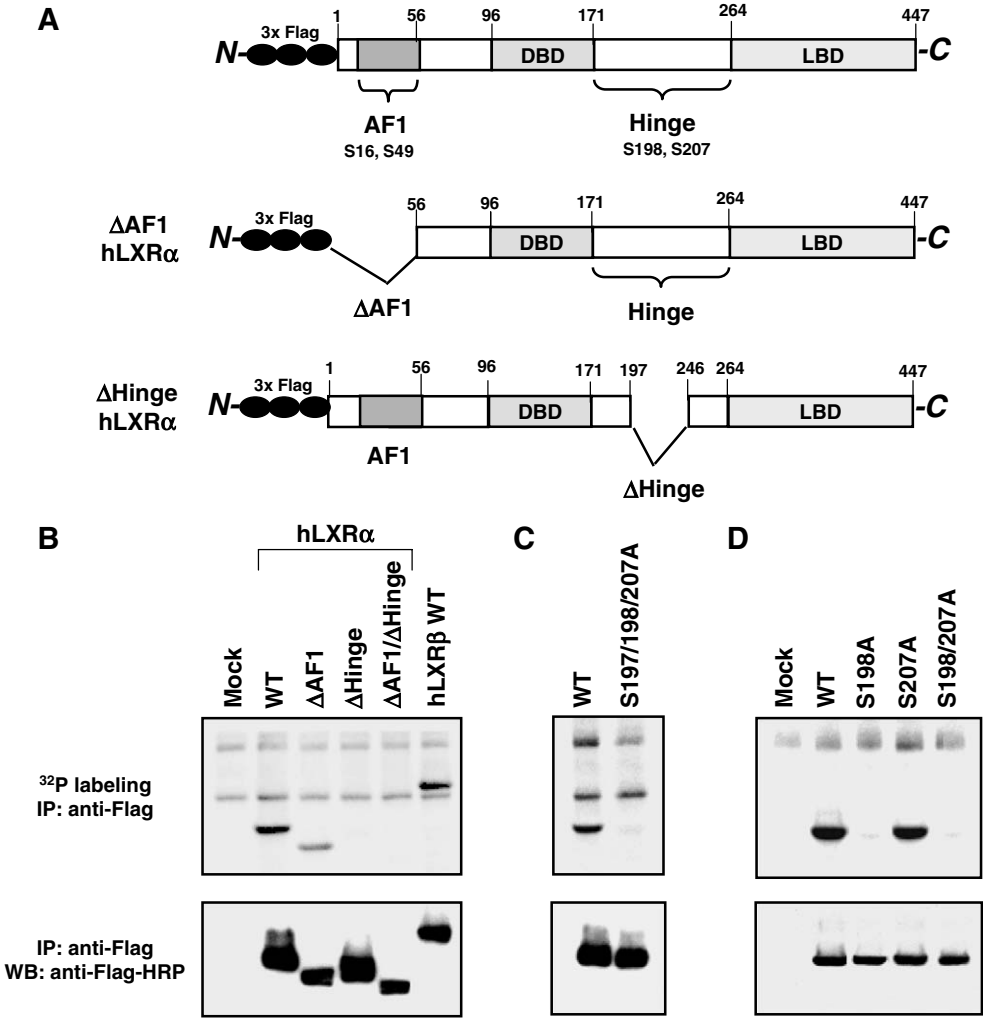

Fig. 3. The major phosphorylation site of LXRα is S198. (A) Schematic representation of the various deletion and point mutants of hLXRα. (B) Phosphorylation of hLXR and the deletion mutants. (C) The triple point mutation of hLXRα (S197, S198, and S207) eliminates phosphorylation of the protein. (D) S198 is the major phosphorylation site for hLXRα.

loaded onto 10% agarose gels, separated by SDS–PAGE, and transferred onto PVDF membranes. Immunoblotting was performed with rabbit anti-phosphoserine antibodies (Zymed). Secondary antibodies were HRP-conjugated anti-rabbit IgG. The signal was detected using standard chemiluminescence techniques in accordance to the manufacturer's protocol (Amersham). In complementary experiments, immunoprecipitation was first performed with a rabbit anti-phosphoserine polyclonal antibody (Zymed) followed by detection with anti-FLAG immunoblotting.

2.8. Immunocytochemistry

Both wild-type hLXRα and hLXRα S198A were cloned in frame to a green fluorescent protein in the pEGFP vector. HEK-293 cells were transiently transfected with these constructs and treated for 8 h with 1 μM T0901317 or vehicle (DMSO). Subcellular localization was determined by fluorescence microscopy (Carl Zeiss Microimaging). Results are typical of $n = 3$ repetitions, where transfection efficiency was ~95–100%.

2.9. In vitro phosphatase assay

FLAG-tagged human LXRs were purified by immunoprecipitation with anti-FLAG antibody beads as described above. Approximately 5–10 μg of the precipitated protein was diluted in 100 μl of phosphatase reaction buffer (20 mM HEPES [pH 7.2]; 150 mM NaCl, 10 mM MgCl2, 0.1% (v/v) Triton X-100, 10% (v/v) glycerol), and then treated with calf intestinal phosphatase (CIP), 5 U, at 30 °C for 1 h. Control reactions did not include the phosphatase. Equal amounts of protein were separated by SDS–PAGE and transferred to PVDF membranes. The protein was detected with anti-FLAG antibodies and visualized by chemiluminescent techniques.

2.10. Metabolic labeling

Two sets of confluent HEK-293 cells were transfected with 4 μg of plasmid encoding the respective proteins. One set of cells was kept in phosphate-free DMEM supplemented with 10% dialyzed FBS (Omega Scientific) and 1.0–2.5 mCi/mL ^{32}P-orthophosphate for 4 h. The other set was not labeled and was harvested for parallel Western blot analysis. Cells were resuspended in 1 mL of lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCL, 1 mM EDTA, 1% Triton X-100) supplemented with a protease inhibitor cocktail (Sigma) and placed on ice for 30 min. After centrifugation, 500 μl of extract was used to immunoprecipitate proteins using anti-FLAG beads. Proteins were separated by SDS–PAGE and transferred to nitrocellulose membranes. Labeled proteins were detected by autoradiography. The parallel, unlabeled proteins were detected by standard Western blot analysis.

3. Results

We used a model system in which epitope-tagged LXRs were stably expressed *in vitro*. The expression of FLAG-tagged proteins was confirmed by immunoblotting with anti-FLAG and anti-LXRα antibodies (Fig. 1A). LXRα migrates as a major band at 50kD, and a second band at ~52 kD, suggestive of a post-translational modification. Induction of the ABCA1 target gene confirmed functional expression of the receptor (Fig. 1B,C). Robust induction of ABCA1 was observed in response to the synthetic LXR agonist T0901317 (Fig. 1B, lane 4). It should be noted that RAW cells do not express LXRα endogenously, but do have basal levels of LXRβ [20]. Therefore, baseline ABCA1 expression and induction by ligand in these cells is expected (Fig. 1B,C).

Next, we investigated whether LXR proteins are phosphorylated in the steady state. Treatment of cell extracts with calf intestinal phosphatase (CIP) from stably transfected RAW cells led to a subtle, but distinct, mobility shift in bands corresponding to both LXRα and LXRβ (Fig. 2A). This observation strongly suggests that both LXRs are phosphorylated proteins. We then used an anti-phosphoserine antibody to show that both LXRα and LXRβ contain phosphoserine residues (Fig. 2B).

The primary amino acid sequences of human LXRα and LXRβ were analyzed by NetPhos 2.0 Server software (http://www.cbs.dtu.dk/services/NetPhos/). Four proline-directed phosphorylation sites were identified by algorithmic prediction: serines 16 and 49 in the AF-1 region and serines 198 and 207 in the hinge region (Fig. 3A). Sequence alignment of human, mouse, and rat LXRs revealed conservation of these sites among the different species. In human LXRα, serine 198 (PRRSSP) and serine 207 (PQLSP) are predicted consensus sites. Strikingly, serine 198 of LXRα is conserved in the alignment with serine 196 of LXRβ. This is the only putative phosphorylation site in the protein with conservation among species and isoforms.

We sought to identify the phosphorylation sites of human LXRα by making deletion mutants. The ΔAF1 mutant lacks amino acids 1–56 of the activating function 1 (AF1) region and the ΔHinge mutant lacks amino acids 197–246 of the hinge region between the DNA binding domain (DBD) and ligand binding domain (LBD) (Fig. 3A). The ΔAF1/ΔHinge mutant lacks both of these regions. We assayed for phosphorylation by labeling with [^{32}P]-orthophosphate. As shown in Fig. 3B, wild-type and deletion constructs express similar protein levels. Deletion of AF1 does not significantly affect phosphorylation, but deletion of the hinge region completely abolishes it. We therefore focused our attention on the hinge region of the pro-

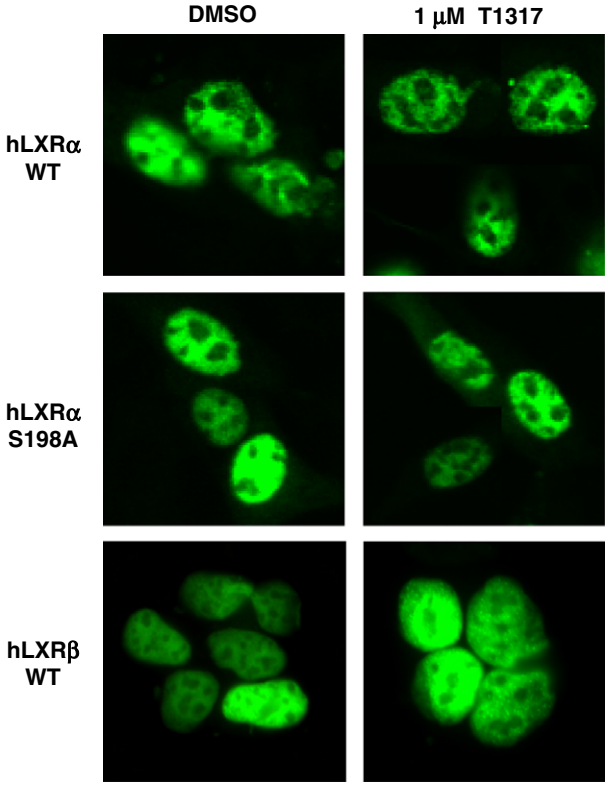

Fig. 4. Localization of GFP-hLXRα in HEK-293 cells. HEK-293 cells transiently expressing GFP-fusion proteins of hLXRα WT, S198A, or hLXRβ were treated with or without LXR ligand (T0901317, 1 μM) for 8 h prior to fixation and DAPI staining. GFP-LXR fusion proteins are visualized by fluorescence microscopy (100×).

tein. Fig. 3B indicates that LXRβ is also phosphorylated, consistent with the conserved serine in both the α (S198) and β (S196) isoforms. In the present study, we chose to focus solely on the site in LXRα.

We made point mutations of the potential phosphorylation sites in the hinge region. Changing serine to alanine prevents phosphorylation at the residue. Double point mutations (S198A, S207A) and a triple mutant (S197A, S198A, S207A) were also made. Each of the point mutants express at comparable levels in HEK-293 cells (Fig. 3C, D lower panels). The triple mutant was unable to be phosphorylated in this assay (Fig. 3C, lane 2), as was the double mutant (Fig. 3D, lane 5). The S207A mutant displayed normal phosphorylation (Fig. 3D, lanes 2 and 4). By contrast, the S198A single point mutation displayed a complete lack of phosphorylation (Fig. 3D, lane 3). We deduce that S198 is the sole phosphorylation site in hLXRα under these conditions.

To investigate whether phosphorylation affects cellular localization, GFP fusion proteins of wild-type hLXRα and the S198A mutant, as well as hLXRβ, were expressed in HEK-293 cells. As shown in Fig. 4, all three constructs localize to the nucleus in the presence and absence of ligand. We conclude that phosphorylation at S198 does not substantially alter the sub-cellular distribution of the protein and that both LXRs are constitutively nuclear (as expected).

To determine if phosphorylation alters the ability of the receptor to activate transcriptional targets, we performed luciferase-based assays using the fatty acid synthase (FAS) pro-

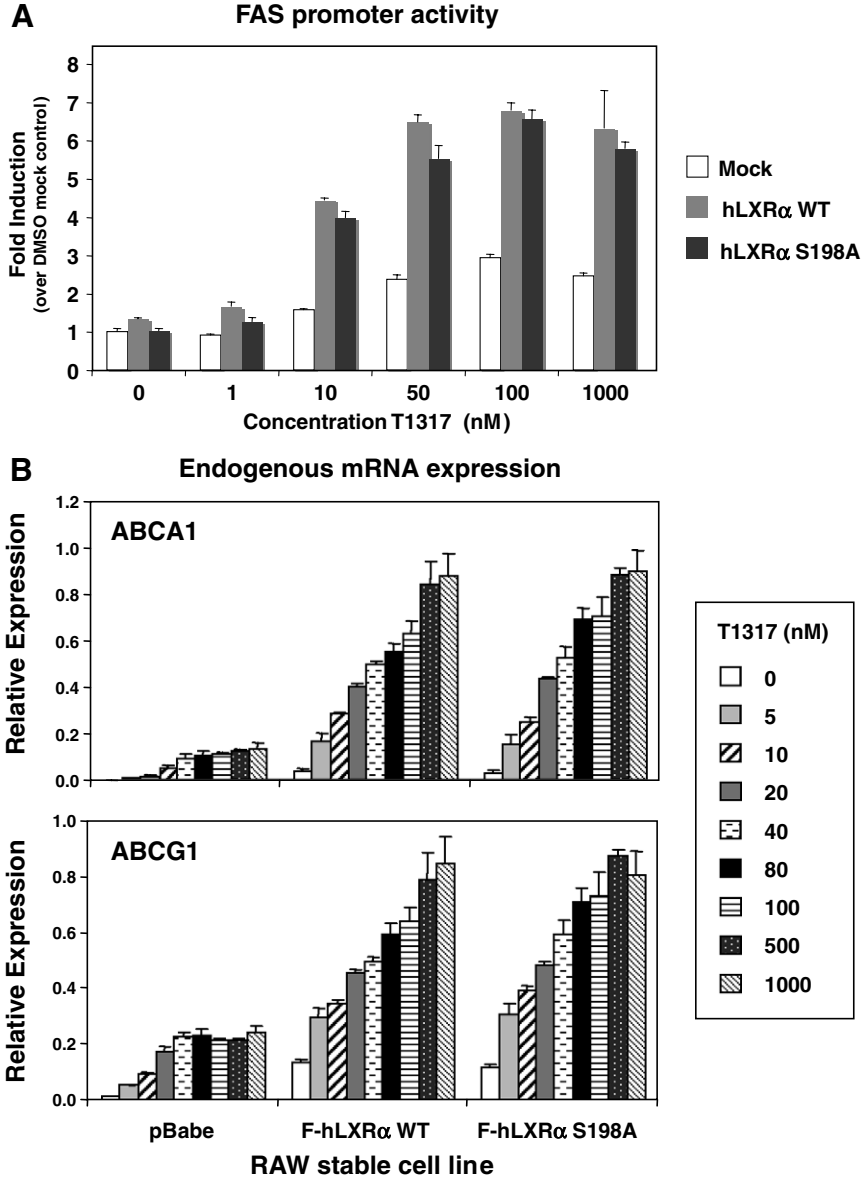

Fig. 5. hLXRα wild-type and S198A mutants have similar transactivating potential. (A) HEK-293 cells expressing hLXRα WT or S198A were assayed for their ability to transactivate an LXRE-containing luciferase reporter plasmid (rFAS-luciferase). Cells were treated with LXR ligand or control media for 24 h. Total luciferase expression was normalized to renilla expression. (B) RAW macrophages stably expressing hLXRα WT or S198A were treated with LXR ligand or control media for 24 h. cDNA was prepared from total cellular mRNA and analyzed by RT-qPCR for LXR target gene expression.

moter [9]. The dose response to synthetic LXR ligand was similar between wild-type and S198A hLXRα (Fig. 5A). There is a consistent trend for the S198A mutant to have less activity at every dose of ligand, but this difference did not reach statistical significance.

To determine if phosphorylation is required for the expression of endogenous target genes, we generated RAW cells stably expressing either hLXRα wild-type or S198A. These cells were treated with increasing doses of LXR ligand and target gene expression was assayed by real-time quantitative PCR. There was no significant difference in ABCA1 or ABCG1 expression (Fig. 5B), suggesting that phosphorylation at S198 is not required for expression of these genes under basal conditions. To rule out possible differences based on clone selection, we repeated the experiments in a second stable pool of hLXRα-S198A expressing RAW cells and again saw no significant difference in target gene expression. LXRs are also known to inhibit several inflammatory genes, including iNOS [7], and in our stably expressing RAW cells, both the wild-type and mutant form of hLXRα inhibited LPS-induced activation of iNOS equally well in response to synthetic ligands (data not shown).

4. Discussion

In addition to regulation in the presence of ligand, nuclear receptor activity is also modified by protein post-translational modifications [21]. Phosphorylation of nuclear receptors appears to be the rule rather than the exception as many examples have been reported previously [12–17,22,23]. Phosphorylation can be ligand-induced, although constitutive phosphorylation sites are also present *in vivo* [23]. Phosphorylation modulates the transcriptional activity of some of these receptors [12,14,24,25]. In this report, we provide the first evidence that both LXRs are phosphorylated and identify a single major phosphorylation site at serine 198 in LXRα. Interestingly, this site is conserved among species and between LXRα and LXRβ. While the present study has focused on LXRα, it seems likely that this same site may be a target for phosphorylation in LXRβ. Serine 198 is characteristic of a MAPK phosphorylation site, but the relevant kinase remains to be definitively identified.

Although LXR phosphorylation may have a role in regulating receptor function, we have not yet been able to determine the purpose of this modification. Surprisingly, LXRα S198A did not alter the ability of the receptor to bind LXR response elements in DNA or to transactivate a reporter gene in transfection assays. It also did not affect the expression of several known LXR target genes in cultured macrophages and did not alter receptor activation by LXR ligand. Specifically, there was no difference in the ability of the mutant LXR receptor to activate the FAS promoter, upregulate the reverse cholesterol transporters ABCA1/G1, or repress LPS-induced stimulation of iNOS. It remains possible that phosphorylation of LXRs may be important for tissue-specific gene expression, response to nutritional or environmental cues, receptor stability, or interactions with other nuclear proteins (e.g. coactivators/corepressors, histones).

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References

[1] Beaven, S.W. and Tontonoz, P. (2006) Nuclear receptors in lipid metabolism: targeting the heart of dyslipidemia. Annu. Rev. Med. 57, 313–329.
[2] Tontonoz, P. and Mangelsdorf, D.J. (2003) Liver X receptor signaling pathways in cardiovascular disease. Mol. Endocrinol. 17, 985–993.
[3] Janowski, B.A., Willy, P.J., Rama-Devi, T., Falck, J.R. and Mangelsdorf, D.J. (1996) An oxysterol signaling pathway mediated by the nuclear receptor LXRα. Nature. 383, 728–731.
[4] Lehmann, J.M., Kliewer, S.A., Moore, L.B., Smith-Oliver, T.A., Oliver, B.B., Su, J.L., Sundseth, S.S., Winegar, D.A., Blanchard, D.E., Spencer, T.A. and Willson, T.M. (1997) Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. J. Biol. Chem. 272, 3137–3140.
[5] Venkateswaran, A., Laffitte, B.A., Joseph, S.B., Mak, P.A., Wilpitz, D.C., Edwards, P.A. and Tontonoz, P. (2000) Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXR alpha. Proc. Natl. Acad. Sci. USA 97, 12097–12102.
[6] Peet, D.J., Turley, S.D., Ma, W., Janowski, B.A., Lobaccaro, J.M., Hammer, R.E. and Mangelsdorf, D.J. (1998) Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. Cell 93, 693–704.
[7] Joseph, S.B., Castrillo, A., Laffitte, B.A., Mangelsdorf, D.J. and Tontonoz, P. (2003) Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. Nat. Med. 9, 213–219.
[8] Liang, G., Yang, J., Horton, J.D., Hammer, R.E., Goldstein, J.L. and Brown, M.S. (2002) Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. J. Biol. Chem. 277, 9520–9528.
[9] Joseph, S.B., Laffitte, B.A., Patel, P.H., Watson, M.A., Matsukuma, K.E., Walczak, R., Collins, J.L., Osborne, T.F. and Tontonoz, P. (2002) Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by liver X receptors. J. Biol. Chem. 277, 11019–11025.
[10] Joseph, S.B., McKilligin, E., Pei, L., Watson, M.A., Collins, A.R., Laffitte, B.A., Chen, M., Noh, G., Goodman, J. and Hagger, G.N., et al. (2002) Synthetic LXR ligand inhibits the development of atherosclerosis in mice. Proc. Natl. Acad. Sci. USA 99, 7604–7609.
[11] Tangirala, R.K., Bischoff, E.D., Joseph, S.B., Wagner, B.L., Walczak, R., Laffitte, B.A., Daige, C.L., Thomas, D., Heyman, R.A. and Mangelsdorf, D.J., et al. (2002) Identification of macrophage liver X receptors as inhibitors of atherosclerosis. Proc. Natl. Acad. Sci. USA 99, 11896–11901.
[12] Hu, E., Kim, J.B., Sarraf, P. and Spiegelman, B.M. (1996) Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARγ. Science 274, 2100–2103.
[13] Shalev, A., Siegrist-Kaiser, C.A., Yen, P.A., Wahli, W., Burger, A.G., Chin, W.W. and Meier, C.A. (1996) The peroxisome proliferator-activated receptor alpha is a phosphoprotein: regulation by insulin. Endocrinology 137, 4499–4502.
[14] Zhang, B., Berger, J., Zhou, G., Elbrecht, A., Biswas, S., White-Carrington, S., Szalkowski, D. and Moller, D.E. (1996) Insulin- and mitogen-activated protein kinase-mediated phosphorylation and activation of peroxisome proliferator-activated receptor gamma. J. Biol. Chem. 271, 31771–31774.
[15] Rousseau, G.G. (1984) Structure and regulation of the glucocorticoid hormone receptor. Mol. Cell. Endocrinol. 38, 1–11.
[16] Kato, S., Endoh, H., Masuhiro, Y., Kitamoto, T., Uchiyama, S., Sasaki, H., Masushige, S., Gotoh, Y., Nishida, E., Kawashima, H., Metzger, D. and Chambon, P. (1995) Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science 270, 1491–1494.
[17] Denner, L.A., Weigel, N.L., Maxwell, B.L., Schrader, W.T. and O'Malley, B.W. (1990) Regulation of progesterone receptor-

mediated transcription by phosphorylation. Science 250, 1740–1743.
[18] Chen, M., Beaven, S.W. and Tontonoz, P. (2005) Identification and characterization of two alternatively spliced transcript variants of human liver X receptor alpha. J. Lipid Res. 46, 2570–2579.
[19] Laffitte, B.A., Joseph, S.B., Walczak, R., Pei, L., Wilpitz, D.C., Collins, J.L. and Tontonoz, P. (2001) Autoregulation of the human liver X receptor alpha promoter. Mol. Cell. Biol. 21, 7558–7568.
[20] Joseph, S.B., Bradley, M.N., Castrillo, A., Bruhn, K.W., Mak, P.A., Pei, L., Hogenesch, J., O'Connell, R.M., Cheng, G., Saez, E., Miller, J. and Tontonoz, P. (2004) LXR-dependent gene expression is important for macrophage survival and the innate immune response. Cell 119, 299–309.
[21] Rosenfeld, M.G., Lunyak, V.V. and Glass, C.K. (2006) Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. Genes Dev. 20, 1405–1428.
[22] Lee, Y.K., Choi, Y.H., Chua, S., Park, Y.J. and Moore, D.D. (2006) Phosphorylation of the hinge domain of the nuclear hormone receptor LRH-1 stimulates transactivation. J. Biol. Chem. 281, 7850–7855.
[23] Orti, E., Bodwell, J.E. and Munck, A. (1992) Phosphorylation of steroid hormone receptors. Endocr. Rev. 13, 105–128.
[24] Blanquart, C., Mansouri, R., Paumelle, R., Fruchart, J.C., Staels, B. and Glineur, C. (2004) The protein kinase C signaling pathway regulates a molecular switch between transactivation and transrepression activity of the peroxisome proliferator-activated receptor alpha. Mol. Endocrinol. 18, 1906–1918.
[25] Lazennec, G., Canaple, L., Saugy, D. and Wahli, W. (2000) Activation of peroxisome proliferator-activated receptors (PPARs) by their ligands and protein kinase A activators. Mol. Endocrinol. 14, 1962–1975.