Biochemical and Biophysical Research Communications 443 (2014) 489–494

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Reciprocal regulation of LXRα activity by ASXL1 and ASXL2 in lipogenesis

Ui-Hyun Park [a], Mi-ran Seong [a], Eun-Joo Kim [b], Wonhee Hur [b], Sung Woo Kim [b], Seung Kew Yoon [c], Soo-Jong Um [a,*]

[a] Department of Bioscience & Biotechnology, Institute of Bioscience, BK21 Graduate Program, Sejong University, Seoul 143-747, South Korea
[b] Department of Molecular Biology, BK21 Graduate Program, Dankook University, Gyeonggi-do 448-701, South Korea
[c] The Catholic University Liver Research Center & WHO Collaborating Center of Viral Hepatitis, The Catholic University, College of Medicine, Seoul 137-701, South Korea

ARTICLE INFO

Article history:
Received 25 November 2013
Available online 7 December 2013

Keywords:
Liver X receptor
ASXL1
ASXL2
Lipogenesis
Liver

ABSTRACT

Liver X receptor alpha (LXRα), a member of the nuclear receptor superfamily, plays a pivotal role in hepatic cholesterol and lipid metabolism, regulating the expression of genes associated with hepatic lipogenesis. The additional sex comb-like (ASXL) family was postulated to regulate chromatin function. Here, we investigate the roles of ASXL1 and ASXL2 in regulating LXRα activity. We found that ASXL1 suppressed ligand-induced LXRα transcriptional activity, whereas ASXL2 increased LXRα activity through direct interaction in the presence of the ligand. Chromatin immunoprecipitation (ChIP) assays showed ligand-dependent recruitment of ASXLs to ABCA1 promoters, like LXRα. Knockdown studies indicated that ASXL1 inhibits, while ASXL2 increases, lipid accumulation in H4IIE cells, similar to their roles in transcriptional regulation. We also found that ASXL1 expression increases under fasting conditions, and decreases in insulin-treated H4IIE cells and the livers of high-fat diet-fed mice. Overall, these results support the reciprocal role of the ASXL family in lipid homeostasis through the opposite regulation of LXRα.

1. Introduction

Liver X receptors (LXRs) are ligand-dependent transcription factors that belong to the nuclear receptor (NR) superfamily [1]. LXRα (NR1H3) is expressed in tissues, such as the liver, spleen, kidney, intestine, and adipose tissue, while LXRβ (NR1H2) is ubiquitously expressed [2,3]. Extensive studies have established that LXRα is a major factor maintaining homeostasis of lipid and cholesterol metabolism by regulating target gene expression [3–5]. LXRα forms a heterodimer with retinoid X receptors (RXRs) and binds to LXR response elements (LXREs) in the promoter of target genes, including sterol regulatory element-binding protein-1c (SERBP-1c), fatty acid synthase (FAS), steroyl CoA desaturase 1 (SCD1), ATP-binding cassette trans porter A1 (ABCA1), and ABCG5 [6–9]. The transcriptional activity of LXRα can be stimulated by natural oxysterol and synthetic ligands, including T0901317 and GW3965 [10], through the concerted dissociation of corepressors (NCoRs) and association of coactivators such as SRC1, ASC2, and PGC-1 [3]. In addition to these classic coregulators, the presence of corepressors of agonist-bound nuclear receptors, including RIP140, LCoR, PRAME, REA, MTA1, NSD1, and COPR1, was recently reported [11]. However, the roles of these corepressors in LXR regulation largely remain unexplored.

Additional sex comb (Asx) was originally studied in *Drosophila* as Enhancer of Trithorax (TrxG) and Polycomb (PcG) group protein [12]. Three paralogs of Asx-like (ASXL) were found in mammals, encoding ASXL1, ASXL2, and ASXL3 [13–15]. Recently, we reported that ASXL1 acts as a dual-function regulator of the retinoic acid (RA) receptor, cooperating with SRC1 for activation or LSD1 and HP1 for repression in the presence of RA [16,17]. Mutations of ASXL1 are often found in humans, mostly linked to chronic myelomonocytic leukemia, myelodysplastic syndromes, and Bohring-Opitz syndrome [18–20]. A recent study using leukemia cells obtained from human patients with ASXL1 mutations showed that ASXL1 is associated with polycomb repressive complex 2, increasing histone H3K27 methylation [21]. Our recent study demonstrated that ASXL1 interacts with ligand-bound PPARγ and inhibits adipogenesis by suppressing PPARγ activity through the reduction of active histone and enrichment of repressive histone in the PPARγ target promoter. In contrast, ASXL2 plays an opposite role in PPARγ-associated adipogenesis [22].

In general, obesity is associated with high adipogenesis in adipose tissues and lipogenesis in the liver. In this regard, our previous findings [22] prompt us to investigate the roles of ASXL1 and

Abbreviations: ASXL, additional sex comb-like; LXRα, liver X receptor alpha; WB, Western blotting; Luc, luciferase; shRNA, small hairpin RNA; KD, knockdown; ChIP, chromatin immunoprecitation; HFD, high fat diet.

* Corresponding author. Fax: +822 3408 4334.
E-mail address: umsj@sejong.ac.kr (S.-J. Um).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.11.124

ASXL2 in the regulation of LXRα activity and lipogenesis in liver cells. Here, we found that both ASXLs interact with ligand-bound LXRα, but oppositely regulate the transcriptional activity of LXRα at the target promoter. Consistently, ASXL1 depletion in H4IIE liver cells promoted palmitate-induced accumulation of lipids, while lipid deposition was impaired in ASXL2- and LXRα-depleted cells. Together with low ASXL1 expression in the livers of high-fat diet (HFD)-fed mice, our data suggest that ASXL1 and ASXL2 oppositely regulate LXRα activity to maintain lipid homeostasis in the liver.

2. Materials and methods

2.1. Cell culture

Rat hepatoma H4IIE cells were maintained in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and an antibiotic–antimycotic (Gibco, Grand Island, NY) in a 5% CO_2 atmosphere at 37 °C. HEK293 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and an antibiotic–antimycotic (Gibco) in a 5% CO_2 atmosphere at 37 °C. For treatment with the LXRα ligand, FBS was pretreated with charcoal.

2.2. Plasmid cloning

All cDNA was constructed according to standard methods and verified by sequencing. DNA sequences encoding murine ASXL1, LXRα, and human ASXL2 were inserted into the modified pcDNA3 vector (Invitrogen, Carlsbad, CA), which harbors the 2 × Flag epitope tag in front of the encoded genes. For the GST-fusion protein, DNA sequences encoding amino acids (aa) 961–1514 of murine ASXL1 or aa 916–1435 of human ASXL2 were inserted into the pGEX4T-1 vector (GE Healthcare, Piscataway, NJ). For His-fusion proteins, murine LXRα full-length cDNA was inserted into the pET15b vector (Novagen, Madison, WI).

2.3. GST pull-down assays

For the GST pull-down assay, GST-fused ASXL1 (aa 961–1514) and ASXL2 (aa 916–1435) were expressed in *Escherichia coli* and purified on glutathione-Sepharose beads (Novagen) by standard methods [23]. GST, GST-ASXL1, or GST-ASXL2 (each 0.2 μg) was mixed with 0.2 μg of His-LXRα and treated with 10 μM of synthetic LXRα ligand T0901317 for 30 min at 30 °C. Pre-equilibrated glutathione-Sepharose beads were added and further incubation was allowed for 1 h. Bound proteins were eluted with 40 μl of 2 × SDS loading buffer by boiling for 10 min and visualized by Western blotting (WB) using an anti-His antibody (Abcam, Cambridge, UK).

2.4. Western blotting (WB) and immunoprecipitation (IP) assays

WB and IP were performed as reported previously [23]. Briefly, H4IIE cells were transfected with the indicated plasmid DNA using the Lipofectamine plus reagent (Invitrogen) overnight and treated with 10 μM of T0901317. For WB, lysates were separated by SDS-PAGE on 6–8% gels, transferred to PVDFs membrane, and blotted with the indicated antibodies. For IP, lysates were pre-cleared by incubating with protein A/G-agarose beads (Santa Cruz Biotechnology, Santa Cruz, CA) for 60 min at 4 °C. Pre-cleared lysates were then mixed with normal IgG, anti-ASXL1 [16], or ASXL2 (Bethyl Laboratories, Montgomery, TX) antibodies. After incubation with protein A/G beads for 4 h at 4 °C, the immune complexes were released from the beads by boiling in sample buffer for 5 min and analyzed by WB using anti-LXRα antibody (Abcam).

2.5. Luciferase reporter gene assays

HEK293 cells were seeded on 12-well plates and transfected with LXRα, LXRE-tk-luciferase reporter, increasing amounts of ASXL1 or ASXL2, and SV40-driven β-galactosidase expression vectors using Lipofectamine (Invitrogen). After transfection for 4 h, cells were fed with DMEM containing 5% charcoal-stripped FBS and incubated overnight in the presence of T0901317 (10 μM). Luciferase activity was measured as described previously [24].

2.6. RNA interference (RNAi)

For depletion of ASXL1, ASXL2, and LXRα using small hairpin RNA (shRNA), the synthetic oligonucleotides were annealed, digested with *Hin*dIII and *Bam*HI, and ligated into the digested pSilencer 2.1-U6 hygro (Ambion, Austin, TX). The H4IIE-derived knockdown stable cell lines were generated by transfecting shRNA expression vectors and selecting resistant colonies against hygromycin (AG Scientific, San Diego, CA) at 0.1 mg/ml. The knockdown efficiency was monitored by WB analysis using anti-ASXL1, anti-ASXL2, and anti-LXRα antibodies. pSilencer hygro luciferase was used as a control (shLuc).

2.7. RNA extraction and reverse-transcription (RT) quantitative-PCR (RT-qPCR)

Total RNA was extracted from H4IIE cells stably depleted with ASXL1, ASXL2, or LXRα using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The cDNA was synthesized with 1 μg of total RNA using MMLV reverse transcriptase and random primers (Invitrogen). Quantitative real-time PCR (qPCR) reactions were performed using the iQ™ SYBR Green Supermix and Icycler CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA). All expression levels were normalized using GAPDH as an internal standard in each well. Fold expression was defined as the fold increase relative to the control.

2.8. Chromatin immunoprecipitation (ChIP) assays

ChIP assay was performed as described previously [16]. H4IIE cells were adapted in 5% charcoal stripped-FBS plus MEM media for 24 h and treated with 10 μM of T0901317 for 30 min. Cross-linked, sheared chromatin complexes were recovered by IP with anti-LXRα, ASXL1, and ASXL2 antibodies. Cross-linking was then reversed according to the protocol from Millipore (Billerica, MA). The DNA pellets were recovered and analyzed by qPCR using a primer pair that encompasses the LXRE of the ABCA1 promoter region [5′-CCCAACTCCCTAGATGTGTC-3′ (forward) and 5′-CCACTCACTCTC GCTCGCA-3′ (reverse)]. Ratios of fold enrichment from each antibody were calculated from Ct values normalized against the Ct of IgG using qPCR. Percentages of input were calculated compared to the input sample used in the qPCR reaction.

2.9. Nile Red staining

H4IIE cells stably depleted with ASXL1, ASXL2, LXRα, or luciferase control (shLuc) were treated with 0.1 mM of sodium palmitate (Sigma–Aldrich, St. Louis, MO) for 24 h. Cells were washed in phosphate-buffered saline (PBS), fixed with 2 ml of 4% formaldehyde in PBS for 5 min, stained with 1 μg/ml Nile Red (Sigma–Aldrich) for 5 min with gentle agitation, and photographed under a fluorescence microscope (Leica Microsystems, Wetzlar, Germany) at a wavelength of 488 nm for excitation and greater than 550 nm for emission.