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Repression of LXRα by a novel member of additional sex comb-like family, ASXL3

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ABSTRACT

Among the members of the additional sex comb-like (ASXL) family, ASXL3 remains unexplored. Here, we showed that ASXL3 interacts with HP1α and LSD1, leading to transcriptional repression. We determined that ASXL3 depletion augments the ligand-induced transcriptional activities of LXRα and TRβ, which were repressed by ASXL3 overexpression. The ligand-dependent interactions of ASXL3 with LXRα and TRβ were demonstrated by the GST pull-down and immunoprecipitation analyses. We confirmed that ASXL3 suppresses the expression of LXRα target genes through its recruitment to the LXR-response elements. Finally, we observed that lipid accumulation in Hep3B cells is downregulated upon ASXL3 overexpression but upregulated upon ASXL3 depletion. Overall, our data suggest that ASXL3 is another corepressor of LXRα, promoting to the regulation of lipid homeostasis.

1. Introduction

Nuclear receptors (NRs) are ligand-activated transcription factors that play critical roles in various biological processes [1]. Liver X receptor alpha (LXRα), a member of the NR superfamily, has emerged as a potential determinant of lipid homeostasis [2]. Expressed in the liver, spleen, kidney, adipose and small intestine [3], LXRα is stimulated by several natural oxysterol and synthetic ligands, including T0901317 and GW3965 [4]. When heterodimerized with retinoid X receptor (RXR), LXRα binds to the LXR-response element (LXRE) in the promoter of target genes that are known to control cholesterol transport and lipogenesis in the liver [5,6]. LXRα target genes involved in cholesterol transport include the ATP binding cassette (ABC) transporters ABCA1, ABCG1, ABCG5, ABCG8, and apolipoprotein E [7,8]. Lipogenesis-associated genes include sterol regulatory element binding protein-1c (SREBP-1c), fatty acid synthase (FAS), acetyl-coenzyme A carboxylase (ACC), and stearoyl-CoA desaturase (SCD-1) [9,10]. The transcriptional activity of LXRα can be regulated through its association with coregulators such as PGC-1β, GPS2, and ACS-2 [10,11]. Similar to LXRα, thyroid hormone receptor (TR) also activates hepatic lipogenesis by stimulating the expression of lipogenic genes, such as FAS and ACC, in the presence of its cognate ligand T_3 [12,13]. The transcriptional activity of TR is diversely regulated depending on the presence of ligand, TR isoforms, the contents of TR response elements, and types of coregulators [14]. In the absence of ligand, TR activity is suppressed by corepressors, such as SMRT and NCoR, whereas it is augmented by coactivators, including steroid receptor coactivator (SRC) and p300/CBP, upon ligand treatment. Recently, a new class of corepressors of ligand-bound nuclear receptors has emerged, including LCoR, PRAME, REA, MTA1, NSD1, and COPR1 [15]. However, the roles of these corepressors in LXRα or TR regulation remain largely unexplored.

Additional sex comb (Asx) was originally identified in *Drosophila* as a member of the Polycomb group of genes that regulate homeotic transformations [16]. Further studies showed that Asx belongs to the enhancer of Trithorax and Polycomb (ETP) group, modulating both the activation and silencing of *Hox* genes [17]. Three Asx homologs have been found in mammals, termed additional sex comb-like 1 (ASXL1), ASXL2, and ASXL3 [18–20]. Consistent with the ETP function of *Drosophila* Asx, we recently reported that mammalian ASXL1 not only enhances but also represses the ligand-induced transcriptional activity of retinoic acid (RA) receptor in a cell type-specific manner by associating with histone-modifying enzymes, such as SRC1 and LSD1 [21,22]. ASXL1 is often mutated in humans; thus, it has been proposed as a risk factor for various diseases, including leukemia [reviewed in [23]]. Recently, the role of ASXL2 was reported in cardiac function [24], adipogenesis [25] and osteoclastogenesis [26].

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While the functions of ASXL1 and ASXL2 have been defined, the function of ASXL3 remains uninvestigated. Here, we determined the role of ASXL3 in regulating the transcriptional activity of nuclear hormone receptor. We found that ASXL3 interacts with LXRα and TRβ in a ligand-dependent manner and suppresses their transcriptional activities. In addition, ASXL3 occupies the promoters of the LXRα target genes and negatively regulates lipid accumulation in Hep3B cells. Overall, our data provide the first evidence of a role of ASXL3 in repressing LXRα activity, which leads to a reduction in lipid accumulation.

2. Materials and methods

2.1. Cell lines and cell culture

H1299 cells were cultured in RPMI 1640 medium, and Hep3B and HEK293 cells were maintained in DMEM medium. Both media were supplemented with 10% heat-inactivated fetal bovine serum (FBS; GenDEPOT, Barker, TX) and antibiotic–antimycotic (Gibco, Grand Island, NY) in a 5% CO_2 atmosphere at 37 °C. For treatment with ligands, FBS was pretreated with charcoal.

2.2. Plasmid construction

All cDNAs were made according to standard methods and verified by sequencing. Partial human ASXL3 cDNA was purchased as a KIAA clone (KIAA1713) from the Kazusa DNA Research Institute (Japan) (AB051500). Full-length ASXL3 cDNA was constructed by joining the N-terminal fragment (amino acids 1–596) of ASXL3 obtained from HEK293 cDNA to the KIAA1713 plasmid. In addition, ASXL3 was inserted into the modified pcDNA3 vector (Invitrogen, Carlsbad, CA), which harbors the 2× Flag epitope tag. Gal4-tagged ASXL3 deletions were subcloned into the pG4MpolyII vector. For antibody production, the DNA sequence encoding amino acids (aa) 1755–1922 of ASXL3 was inserted in the pET15b vector (Novagen, Madison, WI). Human TRα1 and TRβ1 cDNAs obtained from Hep3B cells and mouse LXRα cDNA were inserted into the 2× Flag-tagged pcDNA3 and pET15b vectors, respectively. For the GST-fusion protein, the DNA sequence encoding aa 1457–2248 of ASXL3 was inserted into the pGEX4T-1 vector (GE Healthcare, Piscataway, NJ).

2.3. Transient transfection and luciferase assays

HEK293 cells were seeded on 12-well plates and transfected using Lipofectamine reagent (Invitrogen) with various expression vectors and luciferase reporters as indicated. The CMV-driven-β-galactosidase (gal) expression vector was used as an internal control. After a 4-h transfection, cells were refreshed with medium containing 10% charcoal-stripped FBS and incubated overnight in the absence or presence of cognate ligands (1 μM). Luciferase activity was measured as described previously [25].

2.4. Antibody production

His-tagged ASXL3 (aa 1755–1922) purified from *Escherichia coli* was used to immunize rabbits for polyclonal antibody production. The serum obtained in each boosting step was monitored by Western blotting (WB) analysis. Finally, 5 ml sera were subjected to affinity purification using antigen-coupled agarose beads.

2.5. RNA interference

The sense sequence of a small hairpin (sh) RNA used for ASXL3 knockdown was 5′-AGGATAGAAGATGATCAGTCA-3′. A synthetic duplex harboring additional restriction enzyme sites (*AgeI* and *EcoRI*) was subcloned into the pLKO.1-puro vector (Addgene, Cambridge, MA). A scrambled shRNA sequence purchased from Addgene was used as a negative control (shControl). The knockdown efficiency was monitored by WB analysis using an anti-ASXL3 antibody.

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

WB and IP were carried out as reported previously [22]. Briefly, H1299 cells were transfected overnight with the indicated plasmid DNA using Lipofectamine reagent (Invitrogen), adapted in RPMI 1640 medium supplemented with 10% charcoal-stripped FBS, and treated with cognate ligands for 4 h. For WB, cell lysates were separated by electrophoresis on 6–10% SDS–polyacrylamide gels, transferred to PVDF membranes (Millipore, Billerica, MA), and incubated with the following primary antibodies: anti-Flag (Sigma–Aldrich, St. Louis, MO), anti-His (Applied Biological Materials, Richmond, Canada) and anti-β-actin (Santa Cruz Biotechnology, Santa Cruz, CA) antibodies. For IP, H1299 cells were transfected with the Flag-tagged hTRβ or mLXRα expression vector, and cell lysates (2 mg) were incubated with normal IgG or anti-ASXL3 antibody for 6 h at 4 °C. After additional incubation with protein A/G-agarose beads (Santa Cruz Biotechnology) for 3 h, the beads were washed three times in the absence or presence of ligands (1 μM T_3 or 2 μM T0901317). The immune complexes were released from the beads by boiling in sample buffer and analyzed by WB using the anti-Flag antibody.

2.7. Glutathione S-transferase (GST) pull-down assay

The GST-fused ASXL3 fragment (aa 1457–2248) and His-tagged hTRα, hTRβ and mLXRα were expressed in *E. coli* and purified using glutathione-Sepharose beads (Novagen) and HiTrap™ chelating HP columns (GE Healthcare), respectively. GST or GST-ASXL3 (each 0.2 μg) was mixed with purified His-tagged proteins. The mixtures were incubated for 30 min at 30 °C in the absence or presence of cognate ligands (2 μM). Pre-equilibrated beads with A100 binding buffer (150 mM NaCl, 20 mM Tris–Cl pH 8.0, 0.1% NP-40, and 1 mM EDTA) were added and further incubated for 1 h at 4 °C. Bound proteins were eluted with 2× SDS loading buffer by boiling for 5 min and visualized using WB with an anti-His antibody.

2.8. Reverse transcription and quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from Hep3B cells transfected with shControl or shASXL3 using Isol-RNA lysis reagent (5 PRIME, Gaithersburg, MD) according to the manufacturer's instruction. cDNA was synthesized from 1 μg extracted RNA using M-MLV reverse transcriptase and random primers (Invitrogen). Conventional PCR reactions were performed using primer sets for ASXL3, ABCA1 and SREBP-1c (Table 1). For quantitation, real-time PCR reactions were performed using the iQ™ SYBR Green Supermix and Icycler CFX96 real-time PCR detection system (Bio-Rad). All expression levels were normalized using *GAPDH* as an internal standard.

Table 1
Primer sequences used for RT-PCR.

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
ASXL3	AGGAGTCGTCATGCCCAG	AACAGTCTTGTTTACCATCATT
SREBP-1c	CACTTCATCAAGGCAGACTC	CGGTAGCGCTTCTCAATGGC
ABCA1	AACAGTTTGTGGCCCTTTTG	AGTTCCAGGCTGGGGTACTT
β-Actin	CCAACCGCGAGAAGATGA	CCAGAGGCGTACAGGGA
GAPDH	CTGCACCACCAACTGCTTAGC	GGGCCATCCACAGTCTTCTGG

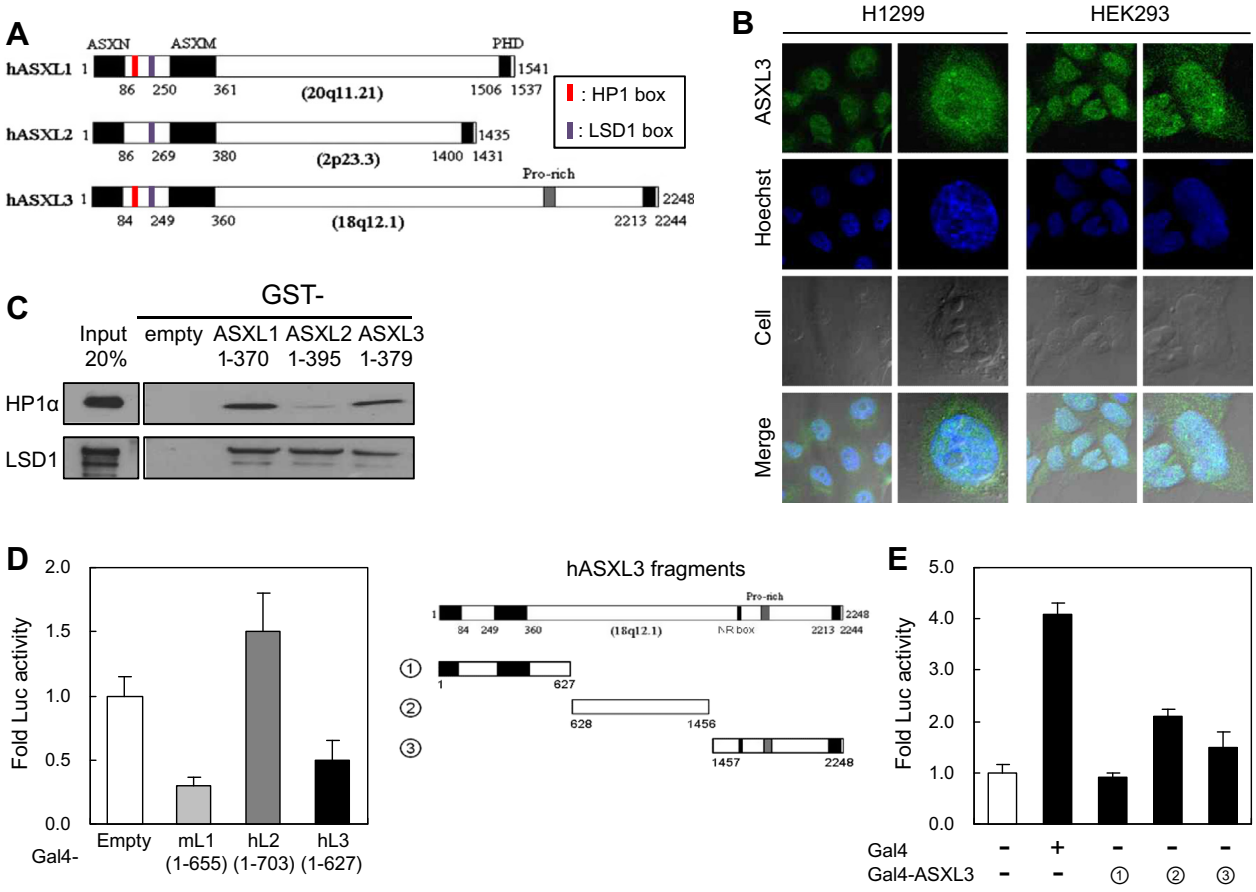

Fig. 1. Cloning and the role of ASXL3 in transcriptional repression. (A) Schematic representation of the human ASXL family. Conserved domains and motifs are shown in black. The HP1 box and NR box are presented in red and blue boxes, respectively. (B) Subcellular location of ASXL3. H1299 and HEK293 cells were immunostained with the ASXL3 antibody and photographed under a fluorescence microscope. Hoechst dye was used to stain the nucleus. (C) Direct interaction of ASXLs with HP1α and LSD1. Purified His-tagged HP1α or LSD1 was incubated with glutathione S-transferase (GST) or the GST-fused N-terminal region of ASXLs. Bound proteins were visualized using Western blotting (WB) with an anti-His antibody. (D) Role of ASXL family members in transcriptional regulation. Gal4 DBD-fused mASXL1 (mL1: aa 1–655), hASXL2 (hL2: aa 1–703), or hASXL3 (hL3: aa 1–627) fragments were transiently introduced into HEK293 cells together with the Gal4-responsive luciferase reporter. (E) Identification of the ASXL3 regions responsible for transcriptional repression. Gal4-fused ASXL3 fragments were transfected into HEK293 cells together with the Gal4-tk-luciferase reporter. The average fold luciferase activity from three independent experiments is shown. Error bars represent the mean ± S.D. ($^{*}p < 0.05$ compared to empty control, $^{**}p < 0.005$, $^{***}p < 0.001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.9. Chromatin immunoprecipitation (ChIP) assay

The ChIP assay was performed as described previously with some modifications [22]. Hep3B cells were transfected with the Flag-mLXRα expression plasmid and adapted in 10% charcoal-stripped FBS plus DMEM for 24 h before treating with T0901317 (2 μM) for 45 min. Cross-linked sheared chromatin complexes were recovered by IP using IgG, anti-ASXL3 and anti-Flag antibodies. Cross-linking was then reversed according to Millipore's protocol. The DNA pellets were recovered and analyzed using conventional PCR or quantitative PCR (qPCR) with primer sets encompassing the LXRE of the ABCA1 promoter region (235 bp) and of the SREBP-1c promoter region (110 bp), respectively. The primers used were as follows: ABCA1, forward, 5′-CCCAACTCCCTA GATGTGTC-3′, and reverse, 5′-CCACTCACTCTCGCTCGCA-3′; SREBP-1c, forward, 5′-CTTTAACGAAGGGGGCGGGAG-3′, and reverse, 5′-GAATGGGGTTGGGGTTACTAGCG-3′. Fold enrichment ratios for each antibody were calculated from qPCR Ct values normalized against IgG Ct values.

2.10. Nile Red staining

To assess the effect of ASXL3 on lipogenesis, Hep3B cells were transfected with 2× Flag-ASXL3 for overexpression and with shRNA for knockdown of ASXL3. Transfected cells were adapted in serum-free DMEM media overnight, treated with 0.1 mM sodium palmitate (Sigma–Aldrich), washed with PBS, and fixed with 4% formaldehyde in PBS for 5 min at room temperature. Cells were then stained with 1 μg/ml Nile Red (Sigma–Aldrich) for 5 min and incubated with 1 μg/ml Hoechst dye for an additional 5 min. After washing with PBS, stained cells were photographed under a fluorescence microscope (Leica Microsystems, Wetzlar, Germany).

2.11. Statistical analysis

Statistical analysis was presented as means ± S.D. of at least 3 independent experiments. Comparisons between multiple groups were presented using paired *t*-tests. *P*-values < 0.05 were considered statistically significant.

3. Results and discussion

3.1. ASXL3 acts as a transcriptional corepressor

Human ASXLs possess various homologous regions, including HP1, LSD1, NR binding motifs and plant homeodomain (PHD) finger, which are conserved among members of the ASXL family (Fig. 1A). A polyclonal antibody was generated and purified to

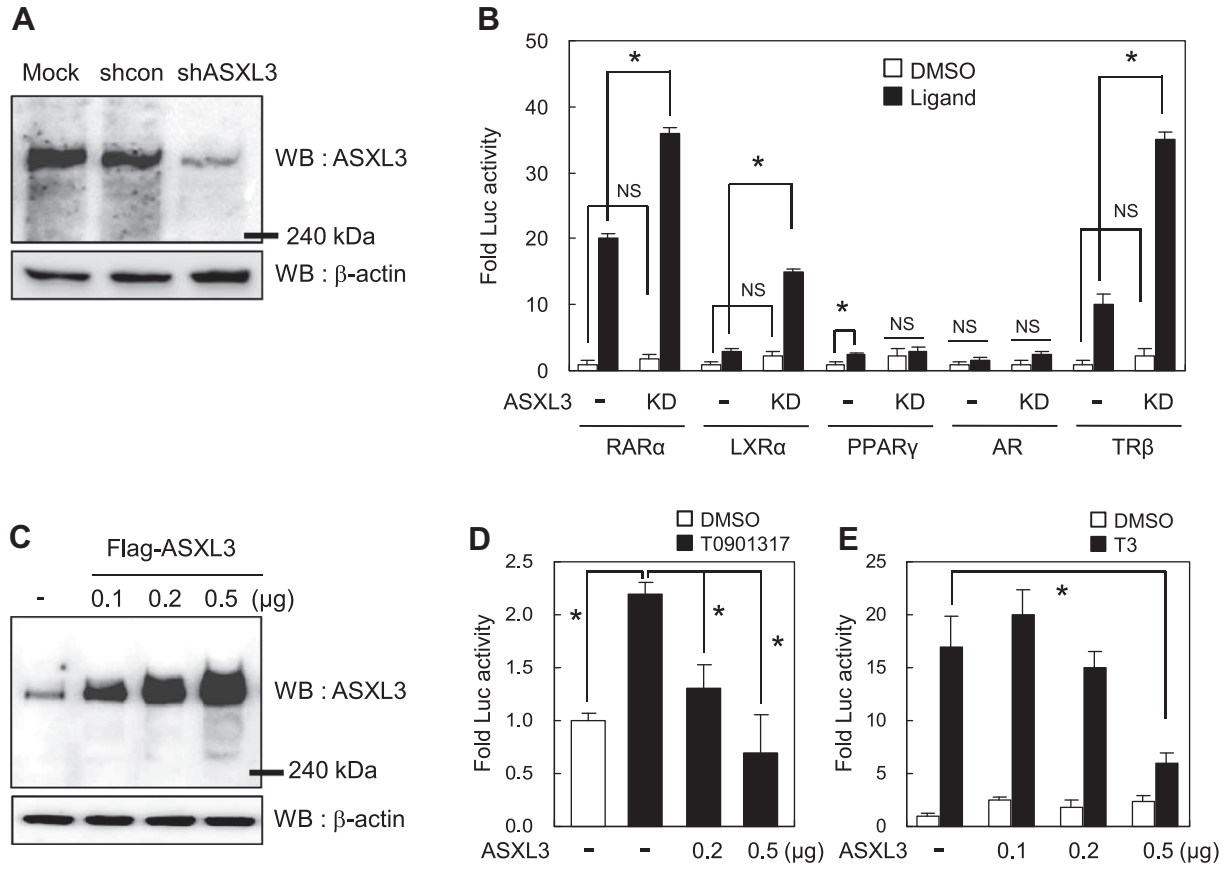

Fig. 2. Effect of ASXL3 on the transcriptional activity of NR. (A) Knockdown of ASXL3. HEK293 cells were transfected with shASXL3. ASXL3 expression was analyzed using WB with the anti-ASXL3 antibody. A scrambled shRNA sequence was used as a negative control (shCon). (B) Effect of ASXL3 knockdown on NR activity. HEK293 cells were cotransfected with NRs and NR-responsive luciferase reporters, as indicated, under mock or ASXL3 knockdown (KD) conditions in the absence (empty bar) or presence (filled bar) of their cognate ligands: all-*trans* RA for RARα; T0901317 for LXRα; rosiglitazone for PPARγ; R1881 for AR; and triiodothyronine (T_3) for TRβ. Error bars represent the mean ± S.D. ($n = 6$, $^*p < 0.05$). NS, not significant. (C) Overexpression of ASXL3. After HEK293 cells were transfected with increasing amounts of Flag-ASXL3, the ASXL3 level was monitored using WB with anti-ASXL3 antibody. (D and E) Effect of ASXL3 overexpression on LXRα (D) and TRβ (E) activity. HEK293 cells were cotransfected with Flag-LXRα (or Flag-TRβ) and increasing amounts of ASXL3 and the LXRE (or TRE-pal)-luciferase reporter in the absence or presence of ligand T0901317 (or T_3). Error bars represent the mean ± S.D. ($n = 3$, $^*p < 0.05$).

investigate the physiological functions of ASXL3 using the His-tagged ASXL3 fragment (aa 1755–1922). The specificity of the purified ASXL3 antibody was determined by blocking with the ASXL3 fragment used to raise the antibody, and no cross reactivity with ASXL1 and ASXL2 (data not shown). As shown in Fig. 1B, ASXL3 was stained mainly in the nucleus in both H1299 and HEK293 cell lines. Previously, we reported that ASXL1 binding to HP1 is critical for RAR repression [22], while ASXL2, which is defective in HP1 binding, enhanced the transcriptional activity of PPARγ [25]. Similar to ASXL1, ASXL3 contains both HP1 and LSD1 binding motifs in its N-terminal region. To validate the interactions, GST pull-down assays were performed using the purified GST-fused N-terminal region of ASXLs and His-tagged HP1α or LSD1 (Supplemental Fig. 1). As indicated in Fig. 1C, both ASXL1 and ASXL3 interact with HP1α and LSD1, whereas ASXL2 interacts only with LSD1. These results prompted us to investigate the role of ASXL3 in transcriptional regulation. Luciferase assays using the Gal4 DNA binding domain (DBD)-fused N-terminal region of ASXLs showed that ASXL3, like ASXL1, possesses a repressive transcriptional function (Fig. 1D). To determine the ASXL3 region responsible for the repression, we generated three ASXL3 fragments fused to Gal4 DBD. Subsequent luciferase assays revealed that all three mediate transcriptional repression, with the strongest repressive activity exerted by the N-terminal region of ASXL3 (Fig. 1E). Further luciferase assays using Gal4-ASXL3 fragments indicated that only the N-terminal region responds to LSD1 and HP1α for repression, presumably led by its interactions (Supplemental Fig. 2). Overall, these data suggest that ASXL3 represses transcription through the associations with HP1 and LSD1.

3.2. ASXL3 targets LXRα and TRβ among NR family members for repression

The repressive role of ASXL3 in transcription, together with our previous findings [22,25], prompted us to investigate whether ASXL3 regulates the transcriptional activity of NR family members. For this purpose, we employed luciferase assays under ASXL3 knockdown and overexpression conditions. Upon knockdown of ASXL3 using shRNA in HEK293 cells, verified by WB (Fig. 2A), we found that ASXL3 depletion leads to significant enhancement of the ligand-induced transcriptional activity of NRs, of which LXRα and TRβ activities were most affected (Fig. 2B). To further confirm the inhibitory effect of ASXL3 on the transcriptional activity of LXRα and TRβ, we carried out luciferase assays in ASXL3-overexpressing HEK293 cells; expression was monitored by WB (Fig. 2C). Upon overexpression of ASXL3, together with Flag-LXRα or Flag-TRβ in the presence of their respective cognate ligands, a significant decrease in ligand-induced luciferase activity of both LXRα (Fig. 2D) and TRβ (Fig. 2E) was observed in a dose-dependent manner. These results suggest that ASXL3 plays a role as a transcriptional corepressor of certain NRs, such as LXRα and TRβ, in

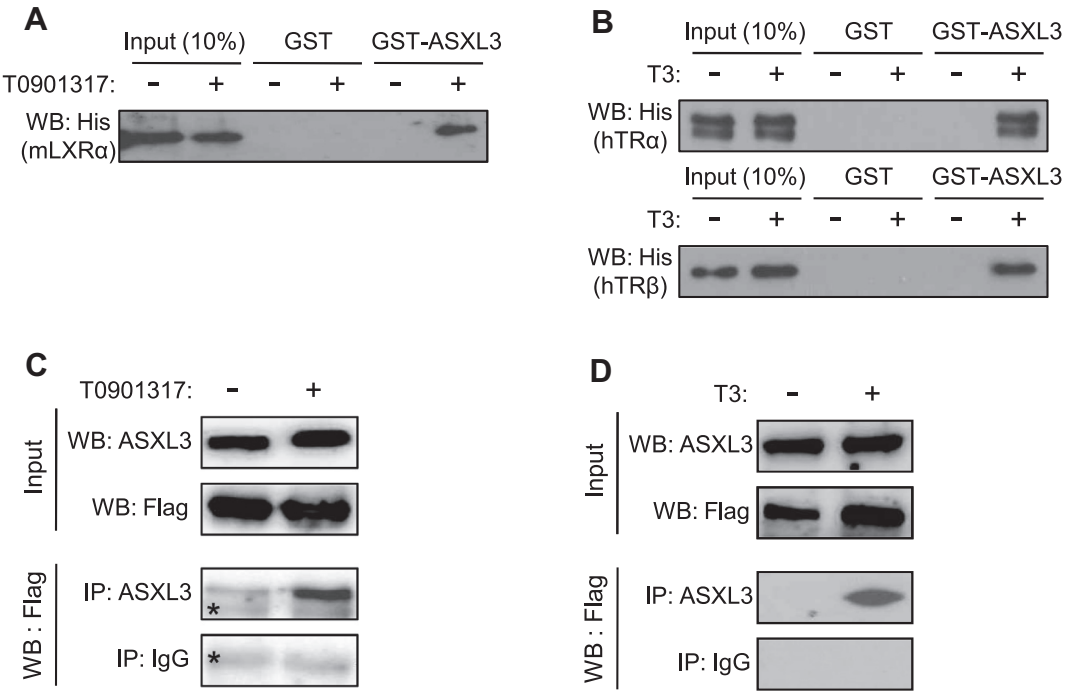

Fig. 3. Interactions between ASXL3 and LXRα or TRs. (A and B) GST pull-down assays. Purified His-tagged mLXRα (A) or hTRs (B) were incubated with GST and the GST-fused ASXL3 fragment. Bound proteins were visualized using SDS–PAGE and WB with the anti-His antibody. (C and D) Immunoprecipitation (IP) assays. H1299 cells were transfected with Flag-mLXRα (C) or Flag-hTRβ (D) expression vector in the absence or presence of ligand. Lysates were pulled down by the IgG control or anti-ASXL3 antibody. Immune complexes were eluted and visualized using WB with an anti-Flag antibody. The asterisk indicates the immunoglobulin heavy chain.

the presence of ligand, and it can be classified as another corepressor of agonist-bound NRs.

3.3. ASXL3 interacts with ligand-bound LXRα and TRβ

To determine the molecular mechanism underlying ASXL3-mediated repression of LXRα and TRβ, the physical interaction between ASXL3 and LXRα or TRβ was investigated *in vitro* and in H1299 cells. Prior to assays *in vitro*, we first performed yeast two-hybrid assays using pBTM116-TRβ and pASV3-ASXL3 deletion mutants as described previously [21] to determine the minimal region of ASXL3 required for TRβ binding. As shown in Supplemental Fig. 3A, ASXL3 fragment (aa 1457–2248) containing the NR box was responsible for T_3-dependent interaction with TRβ. Further, the conserved NR box of ASXL3 was required for the interaction (Supplemental Fig. 3B). For GST pull-down assays, GST-fused ASXL3 (aa 1457–2248) was expressed in *E. coli*, purified, and mixed with His-tagged mLXRα or His-tagged hTRs separately. WB analysis exhibited that ASXL3 interacts directly with LXRα (Fig. 3A) and TRs (Fig. 3B) in ligand-dependent manners. Furthermore, IP assays were performed using lysates prepared from H1299 cells transfected with Flag-mLXRα (Fig. 3C) or Flag-hTRβ (Fig. 3D). IP using anti-ASXL3 antibody followed by WB using anti-Flag antibody demonstrated the interaction between ASXL3 and LXRα or TRβ *in vivo*. These data reveal that ASXL3 interacts directly with agonist-bound LXRα and TRβ.

3.4. ASXL3 negatively regulates lipogenesis

To substantiate the role of ASXL3 in the expression of LXRα-responsive genes and to determine whether ASXL3 is recruited to the LXRα target gene promoters, RT-PCR and ChIP assays were performed using the *ABCA1* and *SREBP-1c* genes in Hep3B cells treated with ligand T09012317. The mRNA expression of both LXRα target genes was enhanced upon depletion of ASXL3 compared with the control (shControl), as determined using conventional RT-PCR (Fig. 4A) and quantitative real-time PCR analysis of ABCA1 and SREBP-1c (Fig. 4B). We further evaluated the effect on ASXL3 knockdown using HEK293 cells and hepatic adenocarcinoma cell line SK-HEP-1. As shown in Supplemental Fig. 4A, the ligand-induced expression of LXRα target gene was augmented upon ASXL3 depletion in HEK293 cells. This effect was also observed in SK-HEP-1 cells (Supplemental Fig. 4B), supporting the negative role of ASXL3 in LXRα function in general.

Prior to ChIP assays, Hep3B cells were transfected with the Flag-LXRα expression vector. Subsequent IP using the indicated antibodies and PCR exhibited that ASXL3, like LXRα, is recruited to the promoters of both LXRα-responsive genes in the presence of T09012317 (Fig. 4C). This ligand-dependent ASXL3 occupancy was further supported by ChIP coupled with quantitative PCR (Fig. 4D). Overall, these data suggest that ASXL3 is recruited to the LXRα target promoter where it then represses LXRα activity in response to ligand. Next, we overexpressed Flag-ASXL3 and depleted endogenous ASXL3 in Hep3B cells and examined the effect of ASXL3 levels on lipid accumulation using Nile Red staining. As shown in Fig. 4E, ASXL3 overexpression reduced intracellular lipid deposition, whereas ASXL3 knockdown augmented it. Taken together, our data suggest that ASXL3 negatively regulates lipogenesis by repressing the transcriptional activity of LXRα, a key mediator of lipogenesis in the liver.

In summary, we first cloned the full-length ASXL3 cDNA, generated a polyclonal antibody against ASXL3, and determined its function associated with NR regulation. Like ASXL1, ASXL3 contains HP1 and LSD1 binding motifs in its N-terminal region and mediates transcriptional repression. Among the NR family members, ASXL3 preferentially targets LXRα and TRβ for repression through direct ligand-dependent interactions as demonstrated *in vitro* and *in vivo*. The biological significance of LXRα regulation by ASXL3 was further determined using RT-PCR, ChIP assays and Nile Red staining in the human hepatoma Hep3B cell line. From these data,

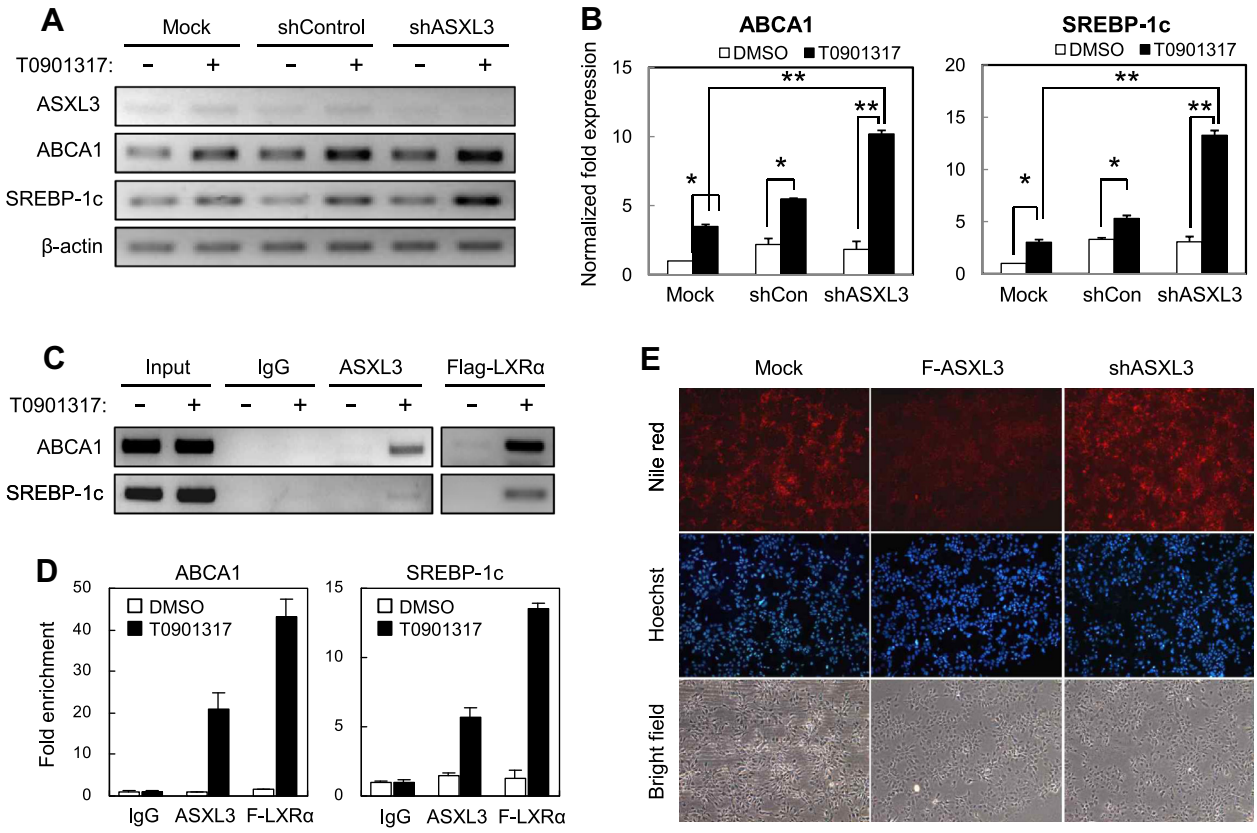

Fig. 4. Negative regulation of LXRα activity by ASXL3 *in vivo*. (A and B) Effect of ASXL3 on the expression of the LXRα target gene. (A) RT-PCR. Total RNA was extracted from Hep3B cells transfected with shControl (scramble) or shASXL3 plasmid and subjected to RT-PCR. Data from one representative experiment of three independent experiments are shown. (B) Effect of ASXL3 knockdown on the mRNA expression of LXRα target genes. Total RNA was extracted from Hep3B cells transfected with shASXL3 in the presence of 2 μM T0901317, reverse transcribed, and subjected to real time qPCR using primer sets specific for *ABCA1* and *SREBP-1c*. Fold-increase in mRNA expression was normalized to the level of *GAPDH* RNA. Data are the averages of three independent experiments (mean ± S.D., $n = 3$, $^{*}p < 0.05$, $^{**}p < 0.005$). (C and D) Ligand-dependent recruitment of ASXL3 to the LXRα-responsive gene promoter. ChIP assays for LXRα target genes, *ABCA1* and *SREBP-1c*, were followed by conventional PCR (C) and by real-time quantitative PCR (D) using Hep3B cells transfected with Flag-LXRα. Inputs indicate PCR products obtained from the chromatin samples prior to IP. Error bars represent the mean ± S.D. ($n = 3$, $^{*}p < 0.05$). (E) Effect of ASXL3 on palmitate-induced lipogenesis. Hep3B cells were transfected with 2× Flag-ASXL3 or ASXL3 shRNA expression vector. Transfected Hep3B cells were treated with 0.1 mM palmitate for 24 h and observed using Nile Red staining. Original magnification ×100. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

we speculate that ASXL3 modulates lipid homeostasis by repressing LXRα activity in response to various dietary conditions. In addition, we observed that ASXL3, through direct interaction, represses the transcriptional activity of TR, an additional mediator of hepatic lipogenesis [12,13]. Further studies are required to determine the role of ASXL3 in TR-mediated lipogenesis in the liver, which may provide insight into the development of therapeutic drugs to address liver-associated metabolic diseases.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.10.074.

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