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HDAC3 interacts with sumoylated C/EBPα to negatively regulate the LXRα expression in rat hepatocytes

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ABSTRACT

The expression changes of liver X receptor alpha (LXRα), histone deacetylase 3 (HDAC3) and CCAAT/enhancer binding protein alpha (C/EBPα) were detected in liver tissues of our high-fat-diet E3 rat model. The aim of this study is to pinpoint the molecular mechanism of HDAC3 and C/EBPα to orchestrate LXRα expression in hepatocytes. We confirmed that *LXRα* and its target genes were negatively regulated by HDAC3 in stable expressed clones with pEGFP-*Hdac3* or shRNA-*Hdac3* vector. However, transient pEGFP-*C/EBPα* plasmid transfection showed an upregulation of LXRα expression and C/EBPα enhanced *LXRα* promoter activity in a dose-dependent manner in CBRH-7919 cells. By using 5′-serial deletion reporter analysis, we identified that fragment from −2881 to −1181 bp of *LXRα* promoter was responsible for C/EBPα binding to the promoter, especially CBS1 and CBS4 were identified essentially by using ChIP and luciferase reporter assay. Co-IP, qRT-PCR and ChIP revealed that HDAC3 interacted with C/EBPα co-regulated LXRα expression. Sumoylation of C/EBPα at lysine 159 was detected in CBRH-7919 cells with transient overexpressed C/EBPα, and Co-IP assay detected that sumoylated C/EBPα interacted with more HDAC3 than C/EBPα K159L mutant. Luciferase reporter assay demonstrated that C/EBPα participated in HDAC3-repressed *LXRα* transcription, and HDAC3 was involved in sumoylated C/EBPα-inactivated *LXRα* activity. Luciferase reporter assay demonstrated that sumoylation of C/EBPα by SUMO-1 directly reversed the activation of C/EBPα on *LXRα* promoter. The results suggested that HDAC3 interacts with sumoylated C/EBPα to negatively regulate the LXRα expression.

1. Introduction

Nuclear receptors (NRs) are ligand-activated transcription factors that regulate diverse biological activities, such as growth, development, reproduction and metabolic homeostasis (Sonoda et al., 2008). The NR family includes 48 members in the human genome, of which regulate lipid and/or glucose homeostasis named metabolic nuclear receptors (MNRs). Among the MNRs, liver X receptor α (LXRα) has appeared as an important regulator of cholesterol metabolism and transportation, glucose homeostasis and inflammation (Calkin and Tontonoz, 2012) and functions as the important regulatory target of metabolic diseases (Sonoda et al., 2008; Calkin and Tontonoz, 2012).

LXRα is mainly expressed in liver; small intestine, kidney, adipose tissue and macrophages (Calkin and Tontonoz, 2010). LXRα can be activated by oxysterols, and executes functions as a heterodimer with retinoid X receptor (RXR). LXRα transactivates a series of genes by binding to the LXR response element (LXRE) in the promoters of target genes, including cholesterol 7α-hydroxylase (CYP7A1), lipoprotein lipase (LPL), fatty acid synthase (FAS), sterol response element binding protein (SREBP)-1c (Chiang et al., 2001; Zhang et al., 2001; Tontonoz et al., 2002; Yoshikawa et al., 2001). Generally, NRs, including LXRα, interact with co-regulators to regulate the expression of their target genes (Hu et al., 2003; Nedumaran et al., 2010; Huuskonen et al., 2004; Laschak et al., 2011; Song

Abbreviations: CBS, C/EBPα binding site; C/EBPs, CCAAT/enhancer binding proteins; ChIP, chromatin immunoprecipitation assay; Co-IP, co-immunoprecipitation; CYP7A1, cholesterol 7α-hydroxylase; FAS, fatty acid synthase; HDAC, histone deacetylase; HFD-MetS, high-fat-diet-induced metabolic syndrome; LPL, lipoprotein lipase; LXRα, liver X receptor alpha; NR, nuclear receptor; NCoR, nuclear receptor corepressor; PTM, post translational modification; qRT-PCR, quantitative real-time PCR; RT, room temperature; SMRT, silencing mediator for retinoid and thyroid receptors; SUMO, small ubiquitin-like modifer; TSS, transcription start site.

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et al., 2012). Co-repressors bind with NRs in the absence of ligand to inhibit the transcriptional activities, and ligand binding with NRs results in conformational changes, release of co-repressors and subsequent recruitment of co-activator to promote the transcription of target genes. The co-regulators exert their functions through the recruitment of chromatin-modified enzymes, which participate in histone acetylation, deacetylation and methylation.

Histone deacetylases (HDACs) catalyze the removal of acetyl from the amino terminal lysine residues of core histones, which generally is associated with gene repression. HDAC3 homologous to yeast Rpd3, belongs to class I HDACs and is expressed ubiquitously. HDAC3 has been identified as the enzymatic component of transcriptional co-repressors complex, which also contains nuclear receptor corepressor (NCoR) and silencing mediator for retinoid and thyroid receptors (SMRT) (Wen et al., 2000; Guenther et al., 2001). The past decade has witnessed increasing evidence that HDAC3 is involved in metabolic activities. Hepatic HDAC3 promotes gluconeogenesis by suppressing lipid synthesis (Sun et al., 2012); fibroblast growth factor 21 is induced to stimulate fatty acid oxidation by inhibition of HDAC3 (Li et al., 2012); and liver-specific deletion of *Hdac3* severely disrupts the metabolic homeostasis (Knutson et al., 2008). Our previous study showed that high constitutive expression of *Hdac3* negatively regulates the *LXRα* expression in livers of E3 rats with high-fat-diet-induced metabolic syndrome (HFD-MetS) (Li et al., 2011), suggesting that modulation of HDAC3 may be an effective approach to mitigate metabolic diseases (Mihaylova et al., 2011). The general mechanism how LXRα regulates its target genes expression has been clarified (Ducheix et al., 2011). But the regulatory mechanism of LXRα itself by co-regulator remains unknown. It has been revealed that suC/EBPδ/HDAC1/HDAC3 complex binds to PPARγ2 promoter and negatively regulates the PPARγ2 transcription in HepG2 cells (Lai et al., 2008). CCAAT enhancer binding protein alpha (C/EBPα), the master adipogenic transcription factor, has been believed as an upstream molecule of LXRα, and regulates mouse LXRα promoter with different consequences in various cell lines (Steffensen et al., 2002).

C/EBPα belongs to C/EBPs family of basic region/leucine zipper (bZIP), which includes six members, C/EBPα, C/EBPβ, C/EBPγ, C/EBPδ, C/EBPε and C/EBPζ. C/EBPα is highly expressed in liver, white adipose tissue, epidermis, lung epithelium and bone marrow myeloid cells (Ramji and Foka, 2002). C/EBPα always forms the homo- or heterodimer with C/EBPs, and exerts pleiotropic effects, such as the differentiation of a variety of cells including adipocytes, hepatocytes, granulocytes, the maintenance of energy homeostasis, and the regulation of constitutive hepatic genes and acute phase response genes. C/EBPα can be modified by post translational modification (PTM) (Yu et al., 2010; Geletu et al., 2007; Behre et al., 2007). C/EBPα has a core sequence I/V-K-X-E, in which the 159 K of the rat is a sumoylation site. Sumoylation of C/EBPα may be a rapid way to limit transcriptional synergy (Geletu et al., 2007; Iniguez-Lluhi et al., 2003) and HDACs participate in sumoylated transcription factors to mediate transcription repression. For examples, HDAC4 inhibits androgen receptor activity through receptor sumoylation (Yang et al., 2011) and also sumoylation of p300 is involved in recruiting HDAC6 (Girdwood et al., 2003) and sumoylated p68 recruits HDAC1 (Jacobs et al., 2007).

C/EBPα and HDAC3 are involved in regulating the LXRα expression in our previous rat model with HFD-MetS, so we hypothesize that there exists a correlation between C/EBPα and HDAC3 and sumoylated C/EBPα may be involved in the inhibition of LXRα expression by HDAC3. Our results indicated that HDAC3 interacts with sumoylated C/EBPα to negatively regulate the LXRα expression in hepatocytes. The findings may provide an intriguing understanding of molecular pathogenesis underlying HDAC3 involvement in metabolic nuclear receptor regulation and interference of HDAC3 may be a new remedy for treatment of metabolic diseases.

2. Materials and methods

2.1. Materials

Rabbit monoclonal antibodies to C/EBPα (ab40761) and HDAC3 (ab32369), and mouse monoclonal antibody to GAPDH (ab8245) were obtained from Abcam Biotechnology, Inc. (Abcam, Cambridge, UK). Goat polyclonal antibody against LXRα (sc-1202) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Secondary antibodies for goat anti-rabbit IgG (H + L), peroxidase conjugated (31460), goat anti-mouse IgG (H + L), peroxidase conjugated (31430) and rabbit anti-goat IgG (H + L), peroxidase conjugated (31402) were from Pierce Biotechnology, Inc. (Pierce, Rockford, USA). All primers were synthesized by Sangon Biotech Co., Ltd. (Sangon, Shanghai, China).

2.2. Stably expressed clone screening and expression vector construction

CBRH-7919 cells, a rat hepatocellular carcinoma cell line, were maintained in RPMI 1640 medium with 10% FBS at 37 °C and 5% CO_2. The pEGFP-*Hdac3* expression vector and pGCsi-shRNA-*Hdac3* vector were constructed and the gene integrity was previously confirmed (Li et al., 2011). The CBRH-7919 cells were seeded in 6-well plates, and grown to 90% confluens; the pEGFP-*Hdac3* vector or pEGFP-N1 vector and pGCsi-shRNA-*Hdac3* interference vector or shRNA-NC vector were transfected into CBRH-7919 cells with Lipofectamine™ 2000 (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. The cells were incubated with the recombinant complexes for 6 h, and then replaced with the complete medium. RPMI 1640 medium with 600 μg/ml G418 was added into the cells 24 h after transfection and this medium was replaced every 3 d for 2 weeks, then the stably expressed clones were screened through limiting dilution analysis. The full length cDNAs of rat C/EBPα and SUMO-1 were generated from E3 rat liver cDNA by PCR and inserted into the pEGFP-N1 eukaryocyte expression vector with double restriction enzyme sites as following: for C/EBPα with sense EcoR I and antisense BamH I and for SUMO-1 with sense Hind III and antisense BamH I. On the basis of pEGFP-*C/EBPα* recombinant, pEGFP-*C/EBPα* K159L expression vector was generated by site-directed mutagenesis through replacement AAG with TTG. The recombinants were identified by PCR, double restriction enzyme reaction and DNA sequencing. The information of GenBank accession number, primers and products is depicted in Table 1.

2.3. RT-PCR and qRT-PCR

Total RNA was isolated with TRIzol reagent (Invitrogen, Carlsbad, USA) and 5 μg RNA was subjected to reverse transcription to synthesize the first strand cDNA. The RT-PCR was used to detect the mRNA expression of *Hdac3* and *LXRα* in clones with stable expression of pEGFP-*Hdac3* or pGCsi-shRNA-*Hdac3* vector. The PCR products were separated by electrophoresis in 1% agarose gels and visualized with ethidium bromide staining. qRT-PCR which performed by iQ5 (Bio-Rad, Hercules, USA) with SYBR® Premix Ex Taq™ II (TAKARA, Dalian, China) was used for detection of mRNA expression of *CYP7A1*, *LPL* and *FAS* in clones with stable expression of pEGFP-*Hdac3* or pGCsi-shRNA-*Hdac3* vector. qRT-PCR was used to examine the mRNA expression of *C/EBPα* in liver tissues of E3 rat model with HFD-MetS and that of *C/EBPα* and *LXRα* in CBRH-7919 cells after transient transfection with pEGFP-N1 and pEGFP-*C/EBPα* plasmid for 48 h. The information of GenBank accession number, primers and products