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Transcriptional regulation of the human Liver X Receptor α gene by Hepatocyte Nuclear Factor 4α

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ABSTRACT

Liver X Receptors (LXRs) are sterol-activated transcription factors that play major roles in cellular cholesterol homeostasis, HDL biogenesis and reverse cholesterol transport. The aim of the present study was to investigate the mechanisms that control the expression of the human LXRα gene in hepatic cells. A series of reporter plasmids containing consecutive 5′ deletions of the hLXRα promoter upstream of the luciferase gene were constructed and the activity of each construct was measured in HepG2 cells. This analysis showed that the activity of the human LXRα promoter was significantly reduced by deleting the −111 to −42 region suggesting the presence of positive regulatory elements in this short proximal fragment. Bioinformatics data including motif search and ChIP-Seq revealed the presence of a potential binding motif for Hepatocyte Nuclear Factor 4 α (HNF-4α) in this area. Overexpression of HNF-4α in HEK 293T cells increased the expression of all LXRα promoter constructs except −42/+384. In line, silencing the expression of endogenous HNF-4α in HepG2 cells was associated with reduced LXRα protein levels and reduced activity of the −111/+384 LXRα promoter but not of the −42/+384 promoter. Using ChiP assays in HepG2 cells combined with DNAP assays we mapped the novel HNF-4α specific binding motif (H4-SBM) in the −50 to −40 region of the human LXRα promoter. A triple mutation in this H4-SBM abolished HNF-4α binding and reduced the activity of the promoter to 65% relative to the wild type. Furthermore, the mutant promoter could not be transactivated by HNF-4α. In conclusion, our data indicate that HNF-4α may have a wider role in cell and plasma cholesterol homeostasis by controlling the expression of LXRα in hepatic cells.

1. Introduction

Liver X receptors (LXRs) are members of the hormone nuclear receptor superfamily of transcription factors with key roles in intracellular and plasma cholesterol homeostasis [1]. LXRs can be activated either by natural products of cholesterol metabolism (oxysterols) or by synthetic compounds such as T0901317 [2]. There are two LXR isoforms, LXRα and LXRβ, sharing almost 80% amino acid identity in their DNA-binding and ligand-binding domains [3]. Experiments in cell cultures and in mice have shown that synthetic LXR ligands promote the efflux of cholesterol from macrophage to extracellular acceptors by up-regulating the genes encoding the membrane lipid transporters ATP Binding Cassette Transporters A1 (ABCA1) and ABCG1 [4]. In the liver, LXRs are strong inducers of hepatic lipogenesis due to the transcriptional up-regulation of Sterol Regulatory Element Binding Protein 1c (SREBP-1c) gene [5]. LXRα is expressed primarily in the liver, intestine, adipose tissue, and macrophages, whereas LXRβ is widely expressed [6]. It has been shown previously that the expression of LXRα in the liver is subject to regulation by dietary fatty acids [7] and by thyroid

Abbreviations: ABCA1, ATP Binsing Cassette Transporter A1; ChIP, Chromatin Immunoprecipitation; ChIP-seq, Chromatin Immunoprecipitation followed by sequencing; DNAP, DNA Affinity Precipitation; HNF-4, Hepatocyte Nuclear Factor 4; H4-SBM, HNF-4 specific binding motif; HRE, Hormone Response Element; LXRs, Liver X Receptors; NR, Nuclear Receptor; PPAR, Peroxisome Proliferator-Activated Receptor; RCT, Reverse Cholesterol Transport; shRNA, Short hairpin RNA; siRNA, Short interfering RNA; SREBP-1c, Sterol Regulatory Element Binding Protein 1c.

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hormone [8]. In macrophages, the expression of LXRα, but not LXRβ, is induced by synthetic PPARγ ligands via a PPAR response element located in the distal region of the mouse LXR promoter [9]. This distal region also contains LXR responsive elements (LXREs) that have been shown to play a role in the autoregulation of the LXRα gene in macrophages (Fig. 1A) [10].

Hepatocyte Nuclear factor 4α (HNF-4α) is an orphan member of the nuclear receptor superfamily that is expressed mainly in the liver but also in kidney, intestine and pancreas [11]. In humans, heterozygous mutations in the HNF-4α gene are associated with an early-onset form of type II diabetes called maturity onset diabetes of the young 1 [12]. Work from our group and others showed that HNF-4α binds to the promoters of various genes involved in lipid and lipoprotein metabolism [13]. HNF-4α can bind to Hormone Response Elements of the direct depeat with one nucleotide in the spacer region (DR-1) type almost exclusively as a homodimer [14]. However, a systematic examination of the DNA binding specificity of HNF-4 using protein-binding microarrays (PBMs) revealed a novel HNF-4-specific binding motif (H4-SBM) having the consensus sequence 5′ xxxxCAAAGTCCA 3′ [14] and by Chromatin Immunoprecipitation followed by sequencing (ChIP-seq) analysis it was shown that this H4-SBM is uniquely bound by HNF-4α in vivo [14].

In the present study we show that the human and the mouse LXRα genes are targets of HNF-4α in hepatic cells. We identified a novel and conserved H4-SBM in the proximal promoter of the human LXRα gene and we validated this site as a true HNF-4α responsive element using *in vitro* and *ex vivo* techniques. Furthermore, we show that silencing of the endogenous HNF-4α gene in hepatocytes was associated with a reduction in protein levels of LXRα and the activity of the LXRα promoter. These data support a novel role of HNF-4α in cell and plasma cholesterol homeostasis through LXRα.

2. Materials and methods

2.1. Plasmid constructions

The luciferase reporter constructs (−2625/+384)-hLXRα and (−3000/+30)-mLXRα, the sh-HNF-4α and sh-control expression vectors and the pMT2-HNF-4α plasmid have been described previoulsy [8,15,16]. All other reporter vectors were generated by standard cloning procedures as described in the Supplement. Oligonucleotides used as primers in PCR cloning or in mutagenesis are shown in Table 1.

2.2. Cell cultures, transient transfections, treatments, and luciferase and β-galactosidase assays

Human embryonic kidney cells (HEK293T) and human hepatoma HepG2 cells were obtained from ATCC and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, L-glutamine, and penicillin/streptomycin at 37 °C in a 5% CO_2 atmosphere. Transient transfections were performed using the $Ca_3(PO_4)_2$ coprecipitation method. For co-transfection experiments with siRNAs, the Attractene transfection reagent (Qiagen) was used according to the manufacturer's instruction. Luciferase assays were performed using the luciferase assay kit from Promega Corp. according to the manufacturer's instructions. Normalization for transfection efficiency was performed by β-galactosidase assays.

2.3. Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) assays were performed as described previously [17] using the SYBR-Green quantitative PCR (KAPA SYBR® FAST qPCR) kit and the StepOnePlus™ Real-Time PCR System (Applied Biosystems) according ot the manufacturer's

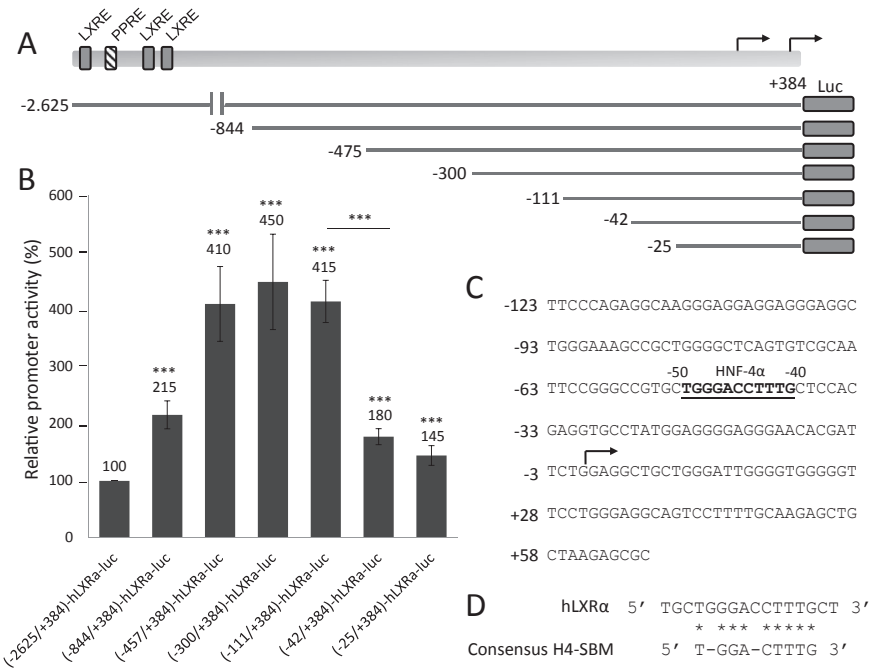

Fig. 1. The human LXRα promoter contains a putative HNF-4α specific binding motif. (A) Schematic representation of the hLXRα promoter. The two transcription start sites, the distal LXREs and the PPRE are depicted. The hLXRα promoter fragments which were used in transfection experiments are shown at the bottom. (B) HepG2 cells were transiently transfected with the hLXRα promoter constructs indicated along with a β-galactosidase expression vector. The normalized relative promoter activity (±SD) was calculated from at least three independent experiments performed in duplicate. (C) Sequence of the proximal hLXRα promoter region spanning nucleotides −123 to +67. The putative HNF-4α binding site in the −50/−40 region of hLXRα promoter is underlined and in bold. (D) Homology of the putative HNF-4 binding site in the −50/−40 region of the human LXRα promoter with the consensus H4-SBM. Identity in the nucleotide sequence is indicated with the asterisks. Key: ***, $p < 0.0001$ by t-testing.

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