Regulation of the human PDZK1 expression by peroxisome proliferator-activated receptor alpha

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Abstract Although PDZK1 is a well-known adaptor protein, the mechanisms for its role in transcriptional regulation are largely unknown. The peroxisome proliferator-activated receptor alpha (PPAR α) is a ligand-activated transcription factor that plays an important role in the regulation of lipid homeostasis. Previously, we established a tetracycline-regulated human cell line that can be induced to express PPAR α and identified candidate target genes, one of which was PDZK1. In this study, we cloned and characterized the promoter region of the human pdzk1 gene and determined the PPAR response element. Finally, we demonstrate that endogenous PPAR α regulates PDZK1 expression.

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1. Introduction

PDZK1 [1], also called C-terminal linking and modulating protein (CLAMP) [2], contains four PDZ-binding domains, which are involved in interactions between many different proteins in a variety of cellular contexts [3]. In the liver, PDZK1 interacts with the scavenger receptor class B type I (SR-BI) and up-regulates SR-BI expression at the protein level [2,4]. SR-BI plays a critical role in lipoprotein metabolism, mainly due to its ability to mediate selective high density lipoprotein (HDL) cholesterol uptake [5]. In PDZK1 knockout mice, hepatic SR-BI protein expression levels were dramatically re-

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Abbreviations: PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; PPRE, peroxisome proliferator responsive element

duced and plasma cholesterol levels were increased [6]. Thus, PDZK1 is important for modulating the lipoprotein metabolism via up-regulation of SR-BI expression [3]. However, the mechanism for transcriptional regulation of PDZK1 expression is largely unknown.

The peroxisome proliferator-activated receptor alpha $(PPAR\alpha)$ is a ligand-activated transcription factor that belongs to the nuclear hormone receptor superfamily [7]. PPARα binds to a direct repeat of two hexanucleotides spaced by one nucleotide, as heterodimers with the retinoid X receptor (RXR) [8]. PPARα activators have been used to treat dyslipidemia, causing a reduction in plasma triglyceride and elevation of HDL cholesterol [9]. Previously, to identify human PPARα responsive target genes, we established a tightly tetracycline (Tet)-regulated human hepatoblastoma cell line that can be induced to express human PPARα (HepG2-tet-off-hPPARα) [10]. Our microarray analyses using HepG2-tet-off-hPPARa cells indicated that $PPAR\alpha$ induced the expression of several genes involved in the β-oxidation of fatty acids and others. Therefore, we think it possible that we could identify the novel targets for PPARa genes from these candidates. Indeed, we observed that PPARa induces human PDZK1 mRNA in these cells, although PDZK1 has not been reported as a direct PPAR target gene.

Here, we examined the relationship between PPAR α and PDZK1. To gain new insights into the transcriptional regulation of PDZK1, we cloned and characterized the promoter region of PDZK1. We demonstrated that PPAR α regulates the expression of PDZK1 via the peroxisome proliferator responsive element (PPRE).

2. Materials and methods

2.1. Plasmid construction

A human PDZK1 promoter fragment spanning -4689 to +38 bp was obtained by means of PCR with a human genomic BAC clone (354F1, Invitrogen) and cloned into PGV-B vector (Toyo Ink) to generate a reporter plasmid (pPDZK1-4689). Deletion constructs were

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-1000 CCAGTGTGGG TGGAAGAGGG <u>GTGAGGTCAC C</u>CAGTCCTCA GCCTGTGGTT <u>TGCTTTGCCT</u> CAGTGCCGGG AGCAATCTCT TGCCC<u>TGATT</u> -911 AP-1 ER alpha 0ct-1-910 TGCATTGTGG GCTGAGGATT CAGGTCAGTG AGAAGGCCAG TGATAGAATT GTCTGTGTGG GTGCTGCTGA TAGGGATCAG CTGCTGATCA -821 GATA3 Myogenin -820 TGGCTAGGGC TAGCGGCTGA GCACACGGGC ACCAGCCTAC TGCTCGGGCA GCAAGGCAGC AGGATGGAGG GGTTTGTAAA AATGCCTTCC -731 -730 ACCTACCC<u>TG CTGGGC</u>TTGC CCGAGAGGCT CTGAGTGTGT GAAGGGGAAG ACAATTATCG CTAGGCTGGA GAATAGAAAG AGCACCATCA -641 -640 GGTGAGAAAG AGAAGAAAAA AAGAGAGGGG AGAGAGTGGA TAAGAAGAAG GAAGCCTGGG ACACAAGAAC AAGGGCTTTG CTTGCTCTAG -551 -550 GGCTTTGCTT GCTAAACTCC TAGGGAGCAC ACAAGTGAGA ATCCAGGGCA GAGGGAAGCA GGATGGGTGC CCTGAGTGCT TGGCCCCATT -461 -460 TCCTGGGGCT TACTGGCCTC GTGGGTGGGA GTTTTATAGG CTACTGCTCC TTGGGGAGGC CTCAGTGATT GAGGATACTC CTGTATGAGC -371 -370 CTCG<u>GCCCTG ACCC</u>CAGGAT GGGGGACTGG AGGATCCTAA CAG<u>GATTGG</u>G GGTTGGTTGG GTGTAACCAA GGTATCTGCC AAAGGGAGAG -281 CCAAT-Box -280 TACCAAGGAA AGCGGAGGCA CCTCCTCCCT GGCTGTCCTT CACCCCCTCT CCCCTCCTTG TTCTCTGGGA GTGGCTGGCG AGCAGCGGCC -191 Sp1 -190 TCCCCGCAGG GCCAGGCAGG TGGGCCAGAG CTTTTGGTTT GCTGAGGTTT GTCAGATTTT CCAGCTCAGG GCCCAGCCAG CTGGCAGGAA -101 C/EBP alpha GCAGGACAGA GGTCACTTGA ATTCAGACCA CATGTCCCTG TTAAATACAT TAGCTTTTAA ATCAATCTTT GTTCAAAGTC CAGTGAGTTG TATA-Box Start of Transcription -10 CAAGCCTAAT GCTCACCTGC AGAGACAGAA TTCCTGAGTG AACGAACAGA GCAGCTCCTC TTCCATCTCC AG-----Intron-----AAATGACCTC CACCTTCAAC CCCCGAGAAT GTAAACTGTC CAAGCAAGAA GGGCAAAACT ATGGCTTCTT CCTGCGAATT GAGAAGGACA CCGAGGGCCA CCTGGTCCGG GTGGTTGAGA AGTGTAGCCC AGCAGAGAAG GCTGGCCTTC AAGATGGAGA CAGAGTTCTT AGGATCAATG PDZK1-2 GTGTCTTTGT GGACAAAGAA GAACATATGC AG-----Intron-----

Fig. 1. Nucleotide sequence of 1-kb 5'-flanking region of the human *pdzk1* gene. The transcription initiation site determined by the CapSite Hunting was assigned as +1. The positions of gene-specific antisense primers used for the CapSite Hunting method are marked by double underlines. The translation initiation codon ATG is indicated by box. Underlined nucleotides are potential transcription factor binding sites proposed by TESS and ERTargetDB.

generated by digestion of pPDZK1-4689. A mutant reporter plasmid, pPDZK1-4689(mut), was constructed by introducing point mutations into the PDZK1 promoter using PCR methods. All constructs were verified by sequencing.

2.2. Cell culture, transfection and reporter gene assays

HepG2 and Huh-7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 7.5% fetal bovine serum (FBS). For ligand treatments, cells were cultured in DMEM supplemented with 10% charcoal/dextran treated FBS (HyClone) and either ligand or DMSO. Luciferase assays were performed as described previously [10]. Briefly, HepG2 cells (4×10⁴ cells/well) were seeded in 96-well plates 14–18 h before transfection. The cells were transfected with 80 ng of the reporter plasmid, 20 ng of phRL-TK (Promega) and either 10 ng of pcDNA3 or pcDNA3-hPPARα expression vector. Twenty-four hours after transfection, the cells were treated with DMSO or 100 μM fenofibric acid. After 24 h, both firefly and *Renilla* luciferase activities were quantified using a Dual-Luciferase® Reporter Assay System (Promega).

2.3. Analysis of the 5'-flanking sequence

The transcription initiation site of the human *pdzk1* gene was determined by the CapSite Hunting method [11] with a human kidney CapSite cDNA® library (Nippon Gene) according to the manufacturer's instructions. Briefly, the first round of PCR was performed using a sense DNA primer complementary to a 38-mer specific oligonucleotide (rOligo) paired with the gene-specific antisense primer 1 (PDZK1-1)

(Fig. 1). Nested PCR was performed using a nested sense DNA primer complementary to rOligo paired with the nested gene-specific antisense primer 2 (PDZK1-2) (Fig. 1). The PCR products were separated, isolated and subcloned into a pGEM®-T Easy vector (Promega). Eleven independent clones were sequenced. Potential binding sites for transcription factors were identified using Transcription Element Search Software (TESS, http://www.cbil.upenn.edu/tess) which is based on TRANSFAC v6.0 database and ERTargetDB, a database for mammalian estrogen receptor alpha (ERα) target promoters [12].

2.4. Electrophoretic mobility shift assay (EMSA)

Human PPARα and human RXRα proteins were prepared using the IMPACT™ -CN system (New England Biolabs). EMSAs were performed as described previously [10]. Supershift assays were performed using anti-human PPARα (H0723, Perseus Proteomics), anti-human RXRα (K8508, Perseus Proteomics) or anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (MAB374, Chemicon International) antibodies. Double-stranded oligonucleotides composed of the following sequences were used for the binding and competition assays: human PDZK1 PPRE wild type, 5'-GAAGCAGACAGAGGT-CACTTG-3'; PDZK1 PPRE mutant, 5'-GAAGCAGACAttGtcGT-CACTTG-3', and rat acyl-CoA oxidase (ACO) PPRE, 5'-GCGGAC-CAGGACAAAGGTCACGTTC-3'.

2.5. Chromatin immunoprecipitation (ChIP)

ChIP assays were performed as described previously [10,13]. Antibodies for PPARα (H-98, Santa Cruz), RXRα (D-20, Santa Cruz) or

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