FEBS Letters 582 (2008) 3884–3888

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

Keisuke Tachibana[a], Naohiko Anzai[b], Chihiro Ueda[a], Tatsuya Katayama[a], Daisuke Yamasaki[a,c], Takayoshi Kirino[a], Rika Takahashi[a], Kenji Ishimoto[a,d], Hidenori Komori[e], Toshiya Tanaka[f], Takao Hamakubo[f], Yukihiko Ueda[g], Hiroyuki Arai[h], Juro Sakai[f], Tatsuhiko Kodama[f], Takefumi Doi[a,c,d,*]

[a] *Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan*
[b] *Department of Pharmacology and Toxicology, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan*
[c] *The Center for Advanced Medical Engineering and Informatics, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*
[d] *Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*
[e] *Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan*
[f] *Laboratory for System Biology and Medicine, Research Center for Advanced Science and Technology, University of Tokyo, 4-6-1 Komaba, Meguro, Tokyo 153-8904, Japan*
[g] *Hirakata Kohsai Hospital, 1-2-1 Fujisaka-Higashi-machi, Hirakata, Osaka 573-0153, Japan*
[h] *Department of Health Chemistry, Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo 113-0033, Japan*

Received 11 September 2008; revised 30 September 2008; accepted 3 October 2008

Available online 26 October 2008

Edited by Ivan Sadowski

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 (PPARα) 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 PPARα 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 PPARα regulates PDZK1 expression.

Keywords: PPARα; PDZK1; PPRE; Target gene

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 reduced 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 (PPARα) 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-hPPARα cells indicated that PPARα 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 PPARα genes from these candidates. Indeed, we observed that PPARα 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 PPARα and PDZK1. To gain new insights into the transcriptional regulation of PDZK1, we cloned and characterized the promoter region of PDZK1. We demonstrated that PPARα 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

*Corresponding author. Address: Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan. Fax: +81 6 6879 8158.
E-mail address: doi@phs.osaka-u.ac.jp (T. Doi).

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

0014-5793/$34.00
doi:10.1016/j.febslet.2008.10.027

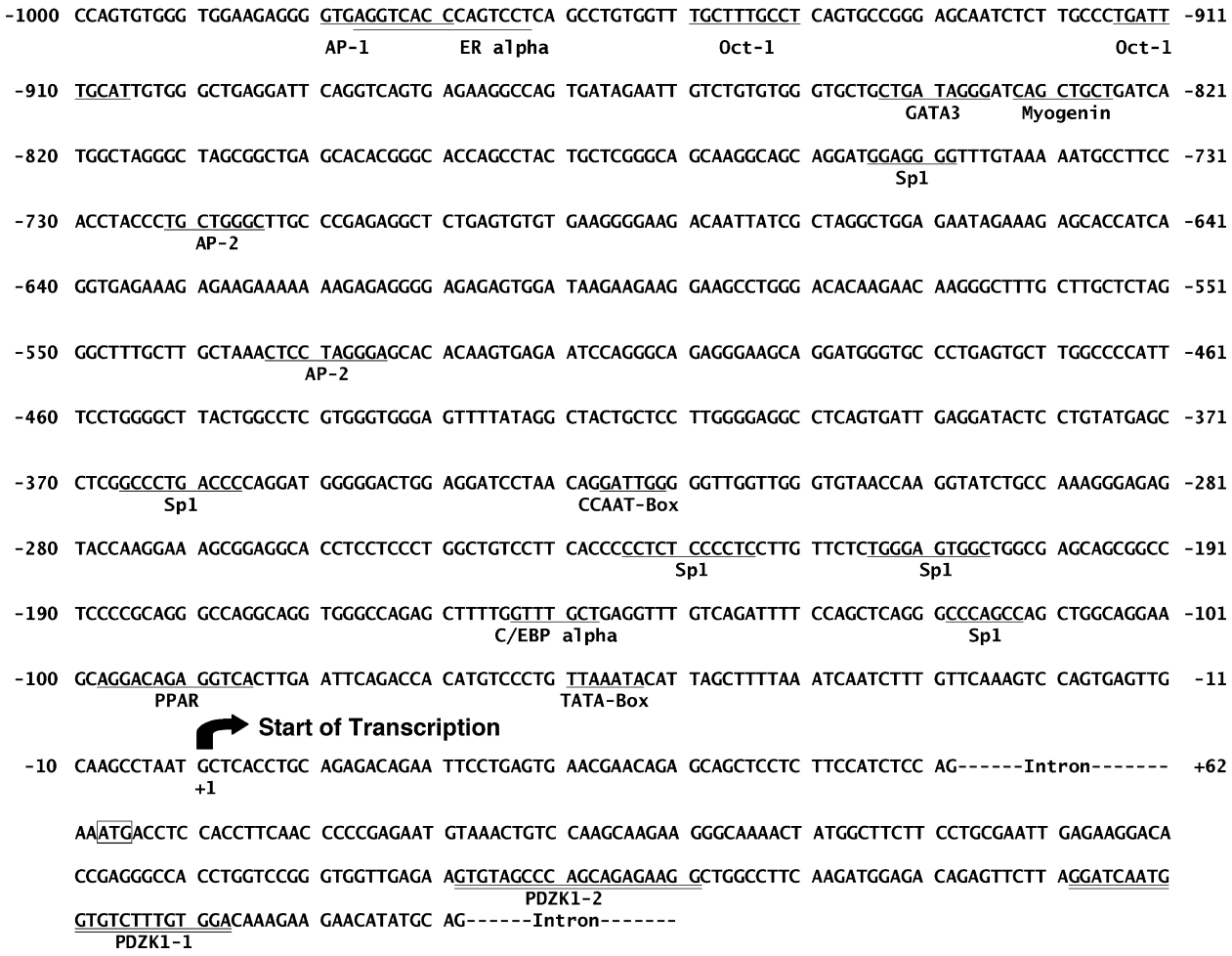

Fig. 1. Nucleotide sequence of 1-kb 5′-flanking region of the human *pdzk1* gene. The transcription initiation site determined by the CapSite Hunting was assigned as +1. The positions of gene-specific antisense primers used for the CapSite Hunting method are marked by double underlines. The translation 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 (4×10^4 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′-GAAGCAGGACAGAGGTCACTTG-3′; PDZK1 PPRE mutant, 5′-GAAGCAGcAttGtcGTCACTTG-3′, and rat acyl-CoA oxidase (ACO) PPRE, 5′-GCGGACCAGGACAAAGGTCACGTTC-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