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PRIC320, a transcription coactivator, isolated from peroxisome proliferator-binding protein complex

Sailesh Surapureddi, Navin Viswakarma, Songtao Yu, Dongsheng Guo, M. Sambasiva Rao, Janardan K. Reddy *

The Department of Pathology, Northwestern University, Feinberg School of Medicine, Chicago, IL 60611, USA

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Abstract

Ciprofibrate, a potent peroxisome proliferator, induces pleiotropic responses in liver by activating peroxisome proliferator-activated receptor α (PPARα), a nuclear receptor. Transcriptional regulation by liganded nuclear receptors involves the participation of coregulators that form multiprotein complexes possibly to achieve cell and gene specific transcription. SDS–PAGE and matrix-assisted laser desorption/ionization reflection time-of-flight mass spectrometric analyses of ciprofibrate-binding proteins from liver nuclear extracts obtained using ciprofibrate–Sepharose affinity matrix resulted in the identification of a new high molecular weight nuclear receptor coactivator, which we designated PRIC320. The full-length human cDNA encoding this protein has an open-reading frame that codes for a 320 kDa protein containing 2882 amino acids. PRIC320 contains five LXXLL signature motifs that mediate interaction with nuclear receptors. PRIC320 binds avidly to nuclear receptors PPARα, CAR, ERα, and RXR, but only minimally with PPARγ. PRIC320 also interacts with transcription cofactors CBP, PRIP, and PBP. Immunoprecipitation–immunoblotting as well as cellular localization studies confirmed the interaction between PPARα and PRIC320. PRIC320 acts as a transcription coactivator by stimulating PPARα-mediated transcription. We conclude that ciprofibrate, a PPARα ligand, binds a multiprotein complex and PRIC320 cloned from this complex functions as a nuclear receptor coactivator.

Keywords: PRIC320; PPARα; Peroxisome proliferators; Ciprofibrate-binding proteins; Quantitative PCR; PBP/MED1; Nuclear receptor coactivator

Peroxisome proliferators constitute a structurally diverse class of chemicals capable of inducing predictably reproducible pleiotropic responses in rat and mouse liver including the development of hepatocellular carcinomas in rats and mice [1]. A high incidence of hepatocellular carcinomas manifests in rats and mice exposed chronically to these synthetic peroxisome proliferators [1]. Peroxisome proliferator-activated receptor α (PPARα), a member of the nuclear receptor superfamily, plays a central role in the induction of peroxisome proliferator-induced cell-specific pleiotropic responses, including the development of liver tumors [1,2]. The PPAR subfamily of nuclear receptors consists of three isotypes, namely PPARα, PPARγ, and PPARβ/δ, and like all other nuclear receptors, PPARs contain a highly conserved DNA-binding domain that recognizes peroxisome proliferator-response elements (PPREs) in the promoter regions of receptor regulated target genes [1–3]. After ligand binding, PPARs heterodimerize with retinoid X receptor (RXR), and the PPAR–RXR heterodimers bind to PPRE to initiate the transcriptional activity [3].

Transcriptional activity of PPARs and other nuclear receptors are regulated by the binding of specific ligands to a given receptor and the orchestrated recruitment of several cofactors [4–7]. These cofactors contribute to the formation of a multisubunit protein complex, variously called thyroid hormone receptor-associated protein (TRAP)/vitamin D receptor-interacting protein/activator-recruited

* Corresponding author. Fax: +1 312 503 8249.
E-mail address: jkreddy@northwestern.edu (J.K. Reddy).

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cofactor/mediator complex that facilitates interaction with RNA polymerase II and the general transcription machinery [4–7].

During the past decade, several nuclear receptor cofactors have been cloned in an attempt to understand the molecular mechanisms by which nuclear receptors achieve transcriptional activation of specific genes in a tissue/cell-specific fashion [4,5,7]. These include p160/steroid receptor coactivator-1 (SRC-1) family with three members (SRC-1, TIF2/GRIP1/SRC2, and pCIP/ACTR/AIB1/RAC3/TRAM1/SRC-3) [4,6,8], CREB-binding protein (CBP) [9], adenovirus E1A-binding protein p300 [5,7], and PPAR-binding protein PBP(TRAP220/DRIP205) [10–12], and PRIP (also known as ASC-2/RAP250/TRBP/NRC) [13–18]. These coactivators contain one or more conserved LXXLL (where L is leucine and X is any amino acid) signature motif(s), which has been found to be necessary and sufficient for ligand-dependent interactions with AF-2 region present in the C-terminal ligand-binding domain of the nuclear receptor [4]. Protein–protein interaction studies suggest that most of these transcription coactivators and coactivator-binding proteins [19,20] are involved in the formation of a multiprotein complex [21]. In an effort to identify specific nuclear proteins that interact with PPARα, we have focused our attention on identifying proteins that bind to this nuclear receptor in the presence of a peroxisome proliferator [22]. We are also generating coactivator gene disrupted mice to study their role in PPARα-mediated target gene transcription [23]. Recently, we reported the identification of a transcriptionally active PPARα-interacting cofactor (PRIC) complex from rat liver nuclear extracts that interacts with full-length PPARα in the presence of ciprofibrate, a synthetic ligand PPARα, and leukotriene B_4, a natural ligand [22]. The PRIC complex revealed ~25 proteins, which were subjected to matrix-assisted laser desorption/ionization reflection time-of-flight (MALDI-TOF) mass spectrometric analyses to establish their identities [21]. We now used this approach to test the feasibility of identifying liver nuclear proteins that bind to peroxisome proliferator ciprofibrate, a potent PPARα ligand. Using this approach, we report the identification and cloning of a new nuclear receptor coactivator in the ciprofibrate-binding protein complex.

Materials and methods

Coupling ciprofibrate to AH-Sepharose 4B. Ciprofibrate was immobilized on AH-Sepharose 4B by carbodiimide reaction, coupling the –COOH group of the ligand to the amino groups (NH_2) on Sepharose 4B with ethylenediamine, a two carbon spacer, as a linker [24,25]. Briefly, cyanogen bromide-activated Sepharose 4B was washed and allowed to swell in cold 1 mM HCl for 30 min. To this activated Sepharose, an equal volume of cold distilled water containing 2 mM of ethylenediamine was added and the reaction was allowed to proceed for 16 h at 4 °C allowing the linkage of spacer [24]. Unreacted cyanogen bromide was blocked with 0.2 M glycine, pH 8.0, and the slurry was washed with distilled water. Ciprofibrate (50 mg) was dissolved in 5 ml dimethylformamide, and the pH of the solution adjusted to pH 4.7 with 1 N HCl and it was then added to activated Sepharose. Five hundred milligrams (2.6 mmol) of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, dissolved in 3 ml of water was added over a 5 min period and the reaction was allowed to continue at room temperature for 20 h. The substituted Sepharose was then washed with 50% dimethylformamide and the ciprofibrate–Sepharose was used for identifying ciprofibrate-binding proteins as below.

Preparation of liver nuclear extracts. Male F 344 rats were fasted overnight to deplete liver glycogen. They were given a single dose of ciprofibrate (250 mg/kg body weight) by gavage and killed 1 h later. Liver nuclei were isolated, broken by homogenization in a Dounce homogenizer on ice, and the extract was stirred for 30 min in a cold room [22]. Nuclear extract was centrifuged at 25,000*g* using a TLA100 ultracentrifuge for 30 min to remove DNA and insoluble material. Supernatant was dialyzed using buffer containing 20 mM HEPES, pH 7.9, 0.1 M KCl, and 20% glycerol with 0.5 mM DTT and 0.5 mM PMSF overnight. The dialysate was centrifuged and the supernatant was stored at −80 °C and used for identifying ciprofibrate-binding proteins.

Affinity pull-down and protein identification. Sepharose beads with immobilized ciprofibrate were blocked with 1% fatty acid-free BSA and washed twice with buffer containing 0.5 M NaCl. Nuclear extract (10 mg protein) was allowed to interact with 50 μl ciprofibrate-immobilized beads overnight at 4 °C. Bound proteins were washed with 40 volumes of buffer containing 0.3 M KCl and 40 volumes with 0.18 M KCl and 10 volumes with buffer containing no salt. Sepharose-bound proteins were analyzed by SDS–PAGE electrophoresis on a 4–15% gradient gel and visualized by silver nitrate or Coomassie blue staining. Selected peptide bands were excised and digested with sequencing grade-modified trypsin. Tryptic fragments were extracted from the gel, co-precipitated with α-cyano 4 hydroxycinnamic acid matrix in acetonitrile, and analyzed by matrix-assisted laser desorption/ionization reflection time-of-flight mass spectrometry (MALDI-TOF). Peptide sequences were identified by matching the ions with the data bank using MS-FIT (Protein Prospector, UCSF) as described [22].

PRIC320 cDNA cloning and plasmid constructs. MALDI-TOF peptides identified by mass spectrometric analysis of one of the high molecular weight ciprofibrate-binding proteins resulted in the identification of a protein, which we designated PRIC320. The peptide sequences matched with human sequence data revealed in KIAA0308 EST clones (AB002306.2; AB002306.3) (obtained from Kazusa, Japan). The cDNA and gene structure analyses suggested that the longest KIAA0308 EST clone (AB002306.3) lacked N-terminal 124 aa. This missing portion was obtained by 5′-RACE PCR with Roche's proprietary 5′-primer (Roche Applied Science) and nested 3′-primers (5′-GCCCTGGGTTGAGATTA TTTCTG-3′ and 5′-AGTCATGGTGTGCCACAAAGC-3′) using total RNA of HeLa and LoVo cell lines (human adenocarcinoma cell line; ATCC #CCL-229). Amplified product was cloned in pCR4-TOPO cloning vector and sequenced. The full-length PRIC320 cDNA sequence, designated PRIC320-1, was deduced. A truncated PRIC320 (ΔPRIC320$^{887–2882}$), designated PRIC320-2, which provides a reading frame of 1996 aa, was constructed by joining the coding sequences of KIAA0308 (AB002306.2) and N-terminal portion of a cDNA (AK022582) obtained from Helix Research Institute, Japan. Plasmids designated pGEX-PPARα, pCMX-PRIP, pCDNA3.1-CBP, and pCDNA3.1-PRIP, GST-PPARγ, GST-RXRα, GST-TRβ, and GST-ERα have been described previously [10,20]. Plasmids encoding ΔPRIC320$^{992–1492}$, ΔPRIC320$^{1493–1993}$, ΔPRIC320$^{1994–2494}$, and ΔPRIC320$^{2495–2882}$ were cloned into pcDNA3.1 and pGEX4 by PCR amplification with hi-fidelity *Taq* polymerase and having suitable restriction sites in the amplifying primers. pGL-PBE-PPRE was constructed using the 3.3 kb promoter of rat peroxisomal *L*-PBE (enoyl-coA hydratase/L-3-hydroxyacyl-co-A dehydrogenase bifunctional enzyme) gene [22]. TNT coupled transcription-translation system (Promega) was used to obtain in vitro translated proteins.

Northern blotting and quantitative real-time PCR. Northern blotting was performed with Clontech human multiple tissue and human cancer cell line blots using PRIC320 cDNA as probe. For quantitative analysis, total RNA from a pooled group of six mice C57BL/6J was prepared from the brain, heart, liver, lung, kidney, skeletal muscle, small intestine, white adipose tissue (WAT), brown adipose tissue (BAT), and testis with