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Human LBP-32/MGR is a Repressor of the P450scc in Human Choriocarcinoma Cell Line JEG-3

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Abstract

Steroid hormones regulate a wide range of physiologic functions in humans. The cholesterol side-chain cleavage enzyme P450scc regulates the initial step of biosynthesis of all steroid hormones. We investigated the expression of P450scc by studying a potential regulator of P450scc, LBP-32/MGR. Using a Northern blot, we found that *LBP-32*/*MGR* mRNA was expressed mainly in the human placenta. Using radiation hybrid mapping, we identified *LBP-32*/*MGR* on human chromosome 2p25. Recombinant LBP-32/MGR protein bound preferentially to a DNA fragment from the promoter of *P450scc* in vitro and exhibited clear nuclear localization in transfected cells. Luciferase reporter gene assays showed that LBP-32/MGR specifically repressed transcriptional activation of the human *P450scc* promoter. Because placental P450scc expression is essential for pregnancy and steroid biosynthesis, the placental expression and transcriptional repressor activity of LBP-32/MGR in JEG-3 cells suggest it has a role as a transcriptional modulator of steroid biosynthesis.

Keywords: LBP-32; Mammalian grainyhead; P450scc; JEG-3; Transcriptional repressor

1. Introduction

The biosynthesis of all steroid hormones is initiated by the conversion of cholesterol to pregnenolone by the mitochondrial cholesterol side-chain cleavage enzyme P450scc (CYP11A) [1,2]. Only 4 tissues in humans produce steroids de novo: adrenal glands, gonads, brain, and placenta [3]. The production of P450scc in adrenal glands and gonads is regulated by steroidogenic factor-1 (SF-1), an orphan nuclear receptor [4]. Despite abundant SF-1 in adrenal glands, gonads, and brain, little or no expression of SF-1 has been detected in placenta [5–7]. These observations imply that SF-1 does not regulate the production of placental P450scc. Moore et al. [1] have shown that a placenta-specific element lies from −155 to −131 region on the *P450scc* promoter. Several transcription factors are found to bind to the −155 to −131 region on the *P450scc* promoter in the placenta [8,9]. Among them, LBP-1b and LBP-9 have been found to function as an activator and a repressor, respectively, that specifically regulate the placental expression of the *P450scc* gene [9]. The expression of LBP-9 is found mainly in JEG-3 cells but not in cell lines from other steroidogenic tissues by RT-PCR [9]. The expression of LBP-1b is found in both steroidogenic tissues and nonsteroidogenic tissues [9]. TReP-132, a zinc finger protein expressing specifically in steroidogenic tissues but not

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restricting to placenta only, is found to interact with coregulator protein CBP/p300 and synergistically increases the *P450scc* promoter activity [10]. All three proteins (LBP-1b, LBP-9, and TReP-132) were found by electrophoretic mobility shift assay (EMSA) to bind to the *P450scc* promoter in the region from −155 to −131 and by transient transfection assay to regulate the expression of the *P450scc* promoter in the human placental cell line JEG-3 [9]. Along with the cloning of human LBP-1b and LBP-9, a third protein (LBP-32) was found to be associated with the *P450scc* promoter by Huang and Miller [9] using the yeast one-hybrid system with the sequence from −155 to −131 from the *P450scc* promoter as a probe. However, no function was described for LBP-32. Later, LBP-32 was renamed by Wilanowski et al. [11] as mammalian grainyhead (MGR). In the literature, LBP-32 is often confused with the laminin binding protein, which has a molecular weight of 32 kDa. Therefore, we refer LBP-32 in this article as ''LBP-32/MGR''. Human LBP-32/MGR protein has high sequence homology to the DNA-binding domain and dimerization domain of the *Drosophila* transcription factor Elf-1 in cuticle-producing tissues (the product of the *grainyhead* gene) or neurogenic element-binding transcription factor (NTF-1) in embryonic central nervous system tissues [12,13]. A 37% homology in the amino acid sequence between human LBP-32/MGR and *Drosophila* Elf-1/NTF-1 was found at the C-terminal end of the LBP-32/MGR. The same region of LBP-32/MGR was also found to contain homologies to *Balanus amphitrite* specific gene BCS-3 [14] and the human α-globin transcription activator CP-2 [15–17]. Since *LBP-32/MGR* cDNA was first cloned using *P450scc* promoter and P450scc is vital for embryogenesis, we decided to further characterize the function of LBP-32/MGR and its involvement with the expression of P450scc. We use human placental cell line JEG-3 as a model because JEG-3 cells are stable transformed human placental cytotrophoblast cell line that retains many characteristics of human cytotrophoblast biology [18,19]. By using purified recombinant LBP-32/MGR protein from baculovirus, we found that LBP-32/MGR is able to bind the promoter of *P450scc* and acts as a repressor to repress the expression of *P450scc* in JEG-3 cells.

2. Materials and methods

2.1. Materials

Cell culture media and sera were purchased from BioWhittaker (Walkersville, MD), Invitrogen (Carlsbad, CA), and Gibco-BRL (Gaithersburg, MD). All oligonucleotide primers were synthesized by Sigma–Genosys (Woodlands, TX) or Integrated DNA Technologies, Inc. (Coralville, IA). Precast polyacrylamide gels and prestained markers were purchased from Bio-Rad (Hercules, CA). Both TALON resin and anti-pentahistidine monoclonal antibody were obtained from BD Biosciences (San Jose, CA). A monoclonal antibody for actin was obtained from Sigma–Aldrich (St. Louis, MO). The polyclonal anti-LBP-32/MGR antibody and anti-BRAK antibody were custom-made by Zymed Laboratories (South San Francisco, CA) against 2 peptides of LBP-32/MGR (residues 19–31, LYPQRRSYTSEDE; and residues 396–408, QIDTYSYNNRSNK) or BRAK (using peptides from both the N-terminal and C-terminal sequences) [20,21], respectively. Hybond-ECL membrane was purchased from Amersham Bioscience (Piscataway, NJ).

2.2. Cell lines and tissues

The Sf9 insect cells, derived from *Spodoptera frugiperda* ovarian cells (Gibco-BRL), were maintained in Grace's insect cell medium supplemented with 10% fetal bovine serum (FBS). Human choriocarcinoma cells JEG-3 from American Type Culture Collection (ATCC, Manassas, VA) were grown in MEM medium supplemented with 10% FBS, 1 mM sodium pyruvate, and 0.1 mM nonessential amino acids. Human kidney cells 293T (ATCC) were maintained in MEM high glucose medium supplemented with 10% FBS and 2 mM L-glutamine.

2.3. Northern blot analysis

A human multiple tissue Northern blot filter was purchased from BD Biosciences. Total RNA of human placenta, adrenal gland, testicle, and ovary (20 μg) were purchased from Ambion (Austin, TX) and applied to a 1% formaldehyde agarose gel. After transferring RNA to Hybond-N+ membrane (Amersham), the membrane and the filter were hybridized with ^{32}P-labeled *LBP-32/MGR* cDNA (nucleotides 1–775 or nucleotides 775–2012), a human *actin* cDNA probe (Ambion, Austin, TX), or a *human glyceraldehyde-3-phosphate dehydrogenase* (GAPDH) cDNA probe (Ambion) at 65 °C for 1–2 h as described previously [22].

2.4. Radiation hybrid mapping of LBP-32/MGR

Radiation hybrid mapping was performed by PCR using the GeneBridge 4 radiation hybrid panel (Research Genetics, Huntsville, AL). The PCR primers were selected to give a different product pattern between positive and negative controls provided in the panel. The primers used were 5′-TGCTGGAGAAAA CAGAGTGC-3′ and 5′-GTCTGGAGTTCGCCTTTGAG-3′. The PCR was begun with a hot start at 94 °C for 30 s, followed by 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, and then a 7-min extension at 72 °C. *Taq* DNA polymerase used in the PCR was purchased from Promega Corporation (Madison, WI).

2.5. Localization of LBP-32/MGR in cells

A 2027-bp cDNA fragment coding for the full-length *LBP-32/MGR* gene was subcloned into pEGFP-C1 vector (BD Biosciences) at the *XhoI/KpnI* sites, which was inframe with the 3′ end of the green fluorescent protein (GFP) cDNA. For transfection, 3×10^4 cells were grown on a 4-well Lab-Tek chambered coverglass (Nalge Nunc International, Rochester, NY) and transfected with 1 μg of LBP-32/MGR in pEGFP-C1 (LBP-32/MGR-GFP) or pEGFP-C1 vector construct using Lipofectamine 2000. After 26 h, confocal microscopy of live cells was performed using an excitation wavelength of 488 nm for GFP and 633 nm for DRAQ5 [23,24] (Biostatus, Leicestershire, UK). Confocal microscopic images of live cells were captured on an Olympus FV500 (ver. #4.3) confocal system with a 60 × 1.4 numerical aperture (NA) objective (Olympus, Melville, NY).

2.6. Polyacrylamide gel electrophoresis and Western blotting

Protein concentrations were estimated by the methods of Bradford using bovine serum ovalbumin as standard. For Western blot, proteins were resolved on 7.5% or 10% SDS-PAGE using a miniprotein II electrophoresis system (Bio-Rad), then transferred to Hybond-ECL membranes (Amersham) using a mini-transblot electrophoretic cell (Bio-Rad) at 22 V for 16 h at 4 °C. After transferring, the membrane was blocked and probed with anti-LBP-32/MGR polyclonal antibody (1:1000 dilution), anti-pentahistidine antibody (1:2000, BD Biosciences), or anti-actin antibody (1:1000 dilution) for 1 h at room temperature as described previously [25].

2.7. Transcription assay

The cDNA encoding the GAL4 DNA-binding domain (residues 1–94) was subcloned into pcDNA3 (Invitrogen), and the resulting pcDNAGAL4 plasmid