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Research Article

Regulation of steroid 5-α reductase type 2 (Srd5a2) by sterol regulatory element binding proteins and statin

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ABSTRACT

In this study, we show that sterol regulatory element binding proteins (SREBPs) regulate expression of Srd5a2, an enzyme that catalyzes the irreversible conversion of testosterone to dihydroxytestosterone in the male reproductive tract and is highly expressed in androgen-sensitive tissues such as the prostate and skin. We show that Srd5a2 is induced in livers and prostate from mice fed a chow diet supplemented with lovastatin plus ezitimibe (L/E), which increases the activity of nuclear SREBP-2. The three fold increase in Srd5a2 mRNA mediated by L/E treatment was accompanied by the induction of SREBP-2 binding to the Srd5a2 promoter detected by a ChIP–chip assay in liver. We identified a SREBP-2 responsive region within the first 300 upstream bases of the mouse Srd5a2 promoter by co-transfection assays which contain a site that bound SREBP-2 *in vitro* by an EMSA. Srd5a2 protein was also induced in cells over-expressing SREBP-2 in culture. The induction of Srd5a2 through SREBP-2 provides a mechanistic explanation for why even though statin therapy is effective in reducing cholesterol levels in treating hypercholesterolemia it does not compromise androgen production in clinical studies.

Introduction

Sterol regulatory element binding proteins (SREBPs) consist of a sub-family of basic-helix–loop–helix transcription factors that play key roles in the regulation of cell lipid homeostasis [1,2]. There are three major SREBP isoforms in mammals that are encoded by two genes. The *Srebf-1* gene produces two overlapping mRNAs that differ only in their specific 5′-terminal exons, where unique 1a and 1c exons give rise to identical proteins except for their unique amino-terminal activation domains [3]. The *Srebf-2* gene produces a single SREBP-2 protein with a potent activation domain similar to SREBP-1a. In cultured cells at least, low cholesterol levels result in membrane release of SREBP-2, whereas low cholesterol and fatty acids trigger release of SREBP-1[4].

Since cholesterol is the precursor of steroid hormones, steroid metabolic synthesis can be affected by SREBPs and conditions where cholesterol is limiting could result in compromised steroid hormone production [5]. A key step in the androgen synthetic pathway converts testosterone into the more biologically active dihydroxytestosterone [6–9]. This step is catalyzed by steroid 5-α reductases, which are membrane-associated NADPH-dependent enzyme that catalyzes the irreversible steroid specific reduction of C^{19} 3-keto-Δ^{4-5} steroid to 5α-reduced metabolites. There are two steroid 5α-reductase isotypes, I (Srd5a1) and II (Srd5a2), in humans and they are composed of 260 and 254 amino acids, respectively, with 47% sequence identity and distinct biochemical properties [9–12].

In mice fed a chow diet supplemented with lovastatin plus ezetimibe (L/E) to limit dietary sterol absorption and decrease

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Abbreviations: Srd5a2, Steroid 5-α reductase type 2; SREBP, sterol regulatory element binding protein; HMG, 3-hydroxy-3-methylglutaryl; ChIP, chromatin immunoprecipitation; qPCR, quantitative PCR; L/E, lovastatin and ezetimibe; LDLR, low density lipoprotein receptor

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endogenous synthesis in the body, nuclear levels of hepatic SREBP-2 are induced [12,13]. Chip studies revealed gene-specific binding of SREBP-2 to known SREBP-responsive genes. In the current study, we searched a genomic promoter-wide ChIP-chip data set for SREBP-2 binding to chromatin from livers of L/E-fed mice and this revealed that the Srd5a2 promoter was bound by SREBP-2. Further studies showed that Srd5a2 gene expression is under the control of SREBP-2 in mouse liver and prostate. These results suggest that steroid hormone production is under the control of SREBP-2 and this regulation could be a key to maintaining androgen activity at normal levels under conditions where cellular cholesterol levels are low. There have been several studies indicating that patients undergoing statin therapy to lower serum cholesterol levels have normal androgen regulated functions [14–16] and the activation of SRD5a2 directly by SREBP-2 provides a molecular explanation for these clinical observations.

Experimental procedures

Animal care

Male 8-week old B6/129 mice were obtained from Taconic and maintained on chow diet (2020X, Harlan Teklad Global) for 1 week with a 12 h light 12 h dark cycle for acclimatization. Then animals were separated into two groups of 6 animals each and one group was maintained on the normal chow diet and the second group was fed the same diet supplemented with a mixture of lovastatin (Mylan Pharmaceuticals Inc., 0.1%, w/w) and ezetimibe (ezetimibe from Merck/Schering-Plough Pharmaceuticals, 0.021%, w/w). After 1 week of feeding, animals were sacrificed by CO_2 asphyxiation in the morning at the end of the dark cycle and tissues were immediately removed for RNA, chromatin and protein extraction as described below.

Chromatin Immunoprecipitation (ChIP) assay

ChIP assays from mouse tissues were performed as previously described [13]. Briefly, livers were pooled and placed in ice-cold PBS solution with a mixture of protease inhibitors. The tissue was minced with a razorblade and processed. Final DNA samples were analyzed by quantitative PCR for SREBP-2 binding to specific gene promoters in triplicate with a standard dilution curve of the input DNA performed in parallel. The qPCR oligonucleotide pairs for the mouse promoters were as follows: Srd5a2, forward 5′-TGAGACCCAGGAGGAATTTG and reverse 5′-CAGTTGTCCATGCTTCTCCA, HMG-CoA reductase, forward 5′-GCTCGGAG ACCAATAGGA-3′ and reverse 5′-CCGCCAATAAGGAAGGAT-3′, L32, forward 5′-ACATTTGCCCTGAATGTGGT and reverse 5′-ATCCTCTTGCCCTGACC TT.

ChIP-chip array

To prepare samples for the ChIP-chip array, after reversing the crosslinking and isolating the ChIP-enriched DNA, samples enriched for SREBP-2, or treated with a non-specific rabbit IgG fraction as a control, along with input DNA were prepared for hybridization to a tiled 1.5-kb mouse promoter array (NimbleGen/Roche) using a random PCR amplification protocol [17]. The results were analyzed by the Signal Map software program from NimbleGen/Roche and Srd5a2 was picked out as having significant SREBP-2 binding.

RNA analyses

Tissues were treated with TRIzol (Invitrogen) and total RNA was extracted according to the manufacturer's instructions. cDNA was synthesized and used as template for qPCR as described [18,19]. All qPCR reactions were performed in triplicate. Primers used for qPCR are as follows: Srd5a2, forward 5′-TTTC CTGGGCGAGATTATTG, reverse 5′-CGCGCAATAAACCAGGTAAT; SREBP-2, forward 5′-ATGCTACAGTTTGTCAGCAATCAAG reverse 5′-TGCTGTTGTTGCCACTG, HMG-CoA reductase, forward 5′-ACCCTGCAGGTCAAACTCTG, reverse 5′-TCACGAACGGTCTCCCTAAC, and L32, forward 5′-ACATTTGCCCTGAATGTGGT and reverse 5′-ATCCTCTTGCCCTGACCTT.

Plasmids

The mouse Srd5a2 promoter construct (−1,500 to +107) was cloned by PCR amplification using mouse genomic DNA as template, followed by recombination with the pDONR2.1 vector according to Gateway technology (Invitrogen). The Srd5a2 construct was then transferred by Gateway technology (Invitrogen) into the luciferase reporter vector p-LUC-GW. All constructs were verified by DNA sequencing. The plasmids, 2×flag pCDNA3.1+ SREBP-2 and pSynSRE-positive control SREBP reporter, have been previously described [3].

Transient transfection in 293T cell and reporter assay

293T cells (2×10^5 cells/well) seeded in 24-well plates were transfected with 200 ng of the Srd5a2 promoter-luciferase reporter and 5 ng of 2×flag pCDNA3.1+ SREBP-2 plasmids using Lipofectamine 2000 reagent (Invitrogen). A pCMV-β-gal expression construct was included in every transfection as a normalization control. Twenty-four hours after transfection, cells were harvested and assayed for luciferase and β-gal activities. Results were computed based on three independent transfections.

Immunoblotting

293T cells were seeded onto 100 mm-culture dishes at 2.5×10^6 cells/dish in DMEM containing 10% FBS and were transfected with 2×flag pCDNA3.1 or 2×flag pCDNA3.1+SREBP-2 and after 24 h, cells were collected by scrapping and were lysed (2% SDS, 5% β-mercaptoethanol, 125 mM Tris–HCl, pH 6.8, 20% glycerol) and 30 μg of total cellular proteins was analyzed by 10% SDS-polyacrylamide gel. For immunodetection of SRD5a2, the polyclonal anti-human Srd5a2 (H-100: sc-20659, Santa Cruz) was used as the primary antibody. The antibody against β-actin (A1978, Sigma) and polyclonal anti-flag antibody (M2; Sigma) were used as the primary antibodies. Using the peroxidase conjugated secondary antibody, the antigen/antibody complex was detected via chemiluminescence (ECL kit, GE) and x-ray film.

LNCaP cells, an androgen-sensitive prostate cancer cell line, were provided by Dr. John Krowleski, UC Irvine. Cells were seeded onto 100 mm dishes at 2×10^6 cells/dish in RPMI1640 supplemented with 10% FCS, 2 mM glutamine, 1 mM sodium pyruvate, and 10 mM HEPES buffer in an atmosphere of 5% CO_2 at 37 °C. The next day, dishes were washed with 1×PBS and refed in the same