Molecular and Cellular Endocrinology 339 (2011) 45–53

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

# Transcriptional regulation of type 11 17β-hydroxysteroid dehydrogenase expression in prostate cancer cells

Mirja Rotinen[a], Joaquín Villar[a,1], Jon Celay[a], Irantzu Serrano[a], Vicente Notario[b], Ignacio Encío[a,*]

[a] *Department of Health Sciences, Universidad Pública de Navarra, Avda. Barañain, 31008 Pamplona, Spain*
[b] *Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC 20057, USA*

ARTICLE INFO

*Article history:*
Received 4 November 2010
Received in revised form 2 March 2011
Accepted 23 March 2011

*Keywords:*
Gene regulation
HSD17Bs
C/EBPα
Sp1
Transcription factors

ABSTRACT

Type 11 hydroxysteroid (17-beta) dehydrogenase (HSD17B11) catalyzes the conversion of 5α-androstan-3α,17β-diol into androsterone suggesting that it may play an important role in androgen metabolism. We previously described that overexpression of C/EBPα or C/EBPβ induced HSD17B11 expression in HepG2 cells but this process was not mediated by the CCAAT boxes located within its proximal promoter region. Here, we study HSD17B11 transcriptional regulation in prostate cancer (PC) cells. Transfection experiments showed that the region −107/+18 is sufficient for promoter activity in PC cells. Mutagenesis analysis indicated that Sp1 and C/EBP binding sites found in this region are essential for promoter activity. Additional experiments demonstrated that ectopic expression of Sp1 and C/EBPα upregulated HSD17B11 expression only in PC cell lines. Through DAPA and ChIP assays, specific recruitment of Sp1 and C/EBPα to the HSD17B11 promoter was detected. These results show that HSD17B11 transcription in PC cells is regulated by Sp1 and C/EBPα.

© 2011 Elsevier Ireland Ltd. All rights reserved.

## 1. Introduction

Androgens are essential for the development and regulation of male sexual characteristics (Hayward and Cunha, 2000). The biological action of androgenic male sex steroid hormones in prostate tissue is mediated by the androgen receptor (AR). Androgens-activated AR is translocated to the nucleus where it binds to androgen response elements located within promoter regions of androgens target genes (Carson-Jurica et al., 1990; McKenna and O'Malley, 2002; Chmelar et al., 2007). This process is well regulated at all stages including at pre-receptor levels which implies the regulation of enzymes that participate in the formation and degradation of 5α-dihydrotestosterone (DHT) (Penning et al., 2008). DHT is the most potent androgen and is responsible for the growth, development and maintenance of the normal secretory function of the prostate (Andersson et al., 1991). In adult males testosterone from the Leydig cells of the testis is converted in the prostate into DHT. On the other hand, it has been described that 5α-androstan-3α, 17β-diol (3α-diol) can be converted into DHT with growth consequences for the prostate (Horst et al., 1975). Deregulation of the process of synthesis and degradation of DHT usually results in benign prostatic hyperplasia and/or prostate cancer (PC). PC is the second leading cause of cancer-related in men (Jemal et al., 2009) and approximately 20% of men with this disease develop metastatic cancer requiring systemic therapy that target androgens production or action.

17β-Hydroxysteroid dehydrogenases (HSD17Bs) are the enzymes responsible for reduction or oxidation of sex hormones, fatty acids and bile acids *in vivo* (Moeller and Adamski, 2009). All require NAD(P(H)) for their activity. Fourteen HSD17Bs have been identified to date and, with the exception of HSD17B5, an aldo-keto reductase (AKR), all of them are short-chain dehydrogenases/reductases (SDRs) (Day et al., 2008). Type 11 17β-hydroxysteroid dehydrogenase (HSD17B11) has been shown to have dehydrogenase activity (Li et al., 1998). It converts 3α-diol into androsterone, which suggests that this enzyme has a role in androgen metabolism (Brereton et al., 2001). HSD17B11, also known as short-chain dehydrogenase/reductase (SDR family) member 8 (DHRS8) and Pan1b, is nearly ubiquitously expressed being highly detected in lung, eyes, liver, pancreas, intestine, kidney, adrenal gland, heart, testis, ovary, placenta and sebaceous gland (Brereton et al., 2001; Chai et al., 2003). Importantly, a recent study demonstrates that HSD17B11 is abundantly expressed in human prostate cancer tissue but not in the normal prostate, suggesting that its expression could be connected with advanced prostate cancer (Nakamura et al., 2009).

HSD17B11 expression has been detected in human steroidogenic cells, including both Leydig and granulosa cells. Importantly,

*Abbreviations:* HSD, hydroxysteroid dehydrogenase; C/EBP, CCAAT enhancer binding protein.

* Corresponding author. Tel.: +34 948166111; fax: +34 948270902.
*E-mail address:* ignacio.encio@unavarra.es (I. Encío).
[1] Current address: Section on Molecular Endocrinology, PDEGEN, NICHD, NIH, Bethesda, MD 20892, USA.

0303-7207/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mce.2011.03.015

the *HSD17B11* 5′-flanking region contains several steroidogenic factor-1 binding sites, but their functionality is still to be demonstrated (Chai et al., 2003). It has also been shown that peroxisome proliferator-activated receptor α (PPARα) regulates HSD17B11 expression in mouse intestine and liver (Motojima, 2004; Yokoi et al., 2007). Although this suggests that HSD17B11 expression is directly regulated by PPARα and its ligand in mouse, promoter sequence up to −1800 bp did not respond to a PPARα ligand in reporter gene assays. Finally, we have described that HSD17B11 is upregulated by C/EBPα and C/EBPβ in the hepatocarcinoma cell line HepG2 but this process was not mediated by the CCAAT boxes located within HSD17B11 proximal promoter (Rotinen et al., 2010).

In order to gain a better understanding of the mechanisms governing the transcriptional regulation of the *HSD17B11* gene and its regulation in prostate cancer, we have cloned and analysed the 5′-flanking region of the human *HSD17B11* gene between −2016 and +18. Serial deletion of the 5′-flanking region of the *HSD17B11* gene identified the region −107/+18 as the minimal promoter. This region included consensus binding sites for C/EBP, Sp1, GATA and NF-κB transcription factors. Mutagenesis analysis showed that the C/EBP and Sp1 sites were essential for promoter activity. Additional experiments indicated that C/EBPα and Sp1 upregulate, bind and are recruited to the promoter of *HSD17B11* gene. Taken together these results indicate that C/EBPα and Sp1 are involved in the transcriptional regulation of the human *HSD17B11* gene in cultured prostate cancer cells.

## 2. Materials and methods

### *2.1. Nucleotide sequence analysis of the HSD17B11 5′-flanking region*

Putative transcription factor binding sites on the 5′-flanking region of the human *HSD17B11* were identified using the MatInspector program found in the Genomatix Software package (Genomatix, Munich, Germany). *Macaca mulatta*, *Pan troglodytes*, *Mus musculus*, *Rattus norvegicus*, *Equus caballus*, *Canis lupus familiars* and *Bos taurus HSD17B11* orthologs were searched for with the Gene2Promoter (Genomatix Software) program.

### *2.2. Generation of human HSD17B11 5′-flanking region/luciferase reporter constructs*

The 5′-flanking region of the human *HSD17B11* gene was isolated by PCR using the oligonucleotides 5′-AAGGTGGGTGGAACAGGAGATCG-3′ (−2016/−1994) and 5′-AAAGAGTAGGGGCGAGAGCAAGG-3′ (+18/−5). PCR products were cloned into a pGEM-T Easy plasmid from Promega (Madison, WI). The −2016/+18 bp insert obtained digesting this construct with *Eco*ICRI and *Nco*I was subcloned into the *Sma*I and *Nco*I sites of the pGL3-Basic vector (Promega) to generate the construct pB11-2016. Construct pB11-344 was obtained from pB11-2016 via PCR with the primers 5′-GTGATAAGCAACCTTCAACTATGA-3′ and 5′-CTTTATGTTTTTGGCGTCTTCCA-3′ and cloning of the PCR product after digestion with *Nco*I into the *Sma*I and *Nco*I sites of the pGL3-Basic vector. Construct pB11-107 was generated by digestion of pB11-2016 with *Nhe*I and circularization of the largest fragment. Constructs pB11-1603 and pB11-1242 were generated from the −2016/+18 pGEM-T Easy, digesting with *Nco*I and either with *Dra*I or *Hinc*II, respectively, and subcloning the resulting fragments into the *Nco*I and *Sma*I sites of the pGL3-Basic vector. The same procedure was used to generate pB11-696, pB11-537 and pB11-150, digesting instead with *Nco*I and either *Bam*HI, *Xho*I or *Hin*dIII and subcloning the resulting fragments into the *Nco*I and *Bgl*II/*Xho*I/*Hin*dIII sites of pGL3-Basic vector. For restriction and ligation Promega and Takara Bio Inc. (Otsu, Shiga, Japan) enzymes were used according to manufacturer's instructions. All constructs were confirmed by sequencing. The C/EBPα and C/EBPβ expression vectors were a gift from Dr. Steven L. McKnight (UT Southwestern Medical Center, Dallas, TX). To generate the Sp1 expression vector, Sp1 mRNA was amplified by RT-PCR and cloned into pcDNA3.1 (Invitrogen, Carlsbad, CA).

### *2.3. Site-directed mutagenesis*

To generate plasmids bearing mutated consensus-binding sequences for transcription factors, mutagenesis experiments were performed using the Quick Change Site-directed Mutagenesis Kit (Stratagene, La Jolla, CA). The chimeric luciferase construct containing the −344/+18 region of the *HSD17B11* promoter was used as a template. Oligonucleotides used were designed with the web based Quickchange Primer Design Program (http://www.stratagene.com/qcprimerdesign) and are listed in Table 1A. The mutated luciferase constructs containing the −107/+18 region were obtained by digestion of the pB11-344 mutated constructions with *Nhe*I and circularization of the largest fragments. All mutations were confirmed by sequencing.

**Table 1**
Sequences of sense and antisense oligonucleotide primers used for (A) site-directed mutagenesis, (B) DNA affinity precipitation assays and (C) amplification of immunoprecipitated chromatin.

(A) Sequences of oligonucleotides used as primers for site-directed mutagenesis experiments

| Oligonucleotides | Sequence (5′–3′) |
|---|---|
| HSD17B11-mC1 (f) | GCAAGCACTGCAGCCCGAGCACTGAGCGAAAGACCAACAAAGCCC |
| HSD17B11-mC1 (r) | GGGCTTTGTTGGTCTTTCGCTCAGTGCTCGGGCTGCAGTGCTTGC |
| HSD17B11-mC2 (f) | GCCTGTGGACACTGTCGGTTATCCTACGAACTTATCCGGTTTAGG |
| HSD17B11-mC2 (r) | CCTAAACCGGATAAGTTCGTAGGATAACCGACAGTGTCCACAGGC |
| HSD17B11-mSp1 (f) | CCAATTGCGAAAGACCAACAAAGAGTAGCCCAGCGGAAGG |
| HSD17B11-mSp1 (r) | CCTTCCGCTGGGCTACTCTTTGTTGGTCTTTCGCAATTGG |
| HSD17B11-mNFKB (f) | TCGGAGTTGTTTTTCTTTGATACGAAAGGCCCTCCTTGCTCTCGCCCCTA |
| HSD17B11-mNFKB (r) | TAGGGGCGAGAGCAAGGAGGGCCTTTCGTATCAAAGAAAAACAACTCCGA |
| HSD17B11-mGATA (f) | GGAACGGTTTCGGAGTTGTTTTTCTTTATTGCGGGAGTTCCTCCT |
| HSD17B11-mGATA (r) | AGGAGGAACTCCCGCAATAAAGAAAAACAACTCCGAAACCGTTCC |

(B) Sequences of oligonucleotides used as probes for DAPA experiments

| Oligonucleotides | Sequence (5′–3′) |
|---|---|
| HSD17B11-CCAAT (f) | BioG/CCCGAGCCAATTGCGAAAGACCAAC |
| HSD17B11-CCAAT (r) | GTTGGTCTTTCGCAATTGGCTCGGG |
| HSD17B11-CCAATmut (f) | BioG/CCCGAGCACTGAGCGAAAGACCAAC |
| HSD17B11-CCAATmut (r) | GTTGGTCTTTCGCTCAGTGCTCGGG |
| HSD17B11-Sp1 (f) | BioG/AACAAAGCCCAGCCCAGCGGAAGGA |
| HSD17B11-Sp1 (r) | TCCTTCCGCTGGGCTGGGCTTTGTT |
| HSD17B11-Sp1mut (f) | BioG/AACAAAGAGTAGCCCAGCGGAAGGA |
| HSD17B11-Sp1mut (r) | TCCTTCCGCTGGGCTACTCTTTGTT |

(C) Sequences of oligonucleotides used as primers for amplification in ChIP experiments

| Oligonucleotides | Sequence (5′–3′) |
|---|---|
| promHSD17B11 (f) | GCAAAGCAGAACTAGGAAGC |
| promHSD17B11 (r) | GCGAGAGCAAGGAGGAAC |

### *2.4. Cell culture, transient transfection and luciferase assay*

LNCaP, PC-3, C4-2, HT-29 and HTB-54 cell lines were obtained from American Type Culture Collection (Manassas, VA). EJ138 cell line was obtained from European Collection of Cell Cultures (Porton Down, UK). LNCaP, C4-2, HTB-54 cells were grown in RPMI 1640 (Invitrogen) medium supplemented with 10% FBS and 100 units/mL of penicillin and 100 μg/mL of streptomycin. PC-3 cells were grown in DMEM-F12 (Invitrogen) supplemented with 10% FBS and 100 units/mL of penicillin and 100 μg/mL of streptomycin. HT-29 and EJ138 cells were grown in DMEM-GlutaMAX (Invitrogen) supplemented with 10% FBS and 100 units/mL of penicillin and 100 μg/mL of streptomycin. Transfections were performed as previously described (Villar et al., 2007). Briefly, cells were seeded in 24-well plates in antibiotic-free medium. The day after, cells were transfected with Lipofectamine LTX Reagent and PLUS Reagent following the Cell-type Specific Transfection Protocols supplied by Invitrogen. After 24 h, cells were harvested and luciferase activities were measured as previously described (Rotinen et al., 2010). Luminescence was measured mixing 20 μL of cell lysate with the Dual-Luciferase Reporter Assay System (Promega) in a Berthold Lumat LB 9501 luminometer (Berthold Technologies, Oak Ridge, TN). Renilla luciferase activity was used to correct the transfection efficiency. Protein concentration was determined by Bradford assay (Bio-Rad Laboratories, Inc., Hercules, CA) using BSA as a standard.

### *2.5. RT and real time quantitative PCR*

Total RNA was isolated with the RNeasy Mini Kit (Qiagen, Valencia, CA). First-strand cDNA synthesis was performed using AffinityScript Multi-

Download English Version:

https://daneshyari.com/en/article/8477800

Download Persian Version:

https://daneshyari.com/article/8477800

Daneshyari.com