Estradiol induces type 8 17β-hydroxysteroid dehydrogenase expression: crosstalk between estrogen receptor α and C/EBPβ

Mirja Rotinen, Jon Celay, Marta M Alonso*, Aranzazu Arrazola, Ignacio Encio and **Joaquin Villar**

Department of Health Sciences, Universidad Pública de Navarra, Avda. Barañain, 31008 Pamplona, Spain

(Correspondence should be addressed to J Villar who is now at National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20892, USA; Email: villarj@mail.nih.gov)

*(M M Alonso is now at the Department of Neuro-Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.)

Abstract

Hydroxysteroid (17-beta) dehydrogenase (HSD17B) are the enzymes responsible for the reversible interconversion of 17-hydroxy and 17-keto steroids. The human and mouse type 8 17β-HSD (HSD17B8) selectively catalyze the conversion of estradiol (E2) to estrone (E1). We previously described that HSD17B8 is transcriptionally regulated by C/EBPβ, and that C/EBPβ is bound to CCAAT boxes located at −5 and −46 of the transcription start site in basal conditions in HepG2 cells. Furthermore, ectopic expression of C/EBPβ transactivated the HSD17B8 promoter activity. Here, we show that HSD17B8 expression is up-regulated in response to E2 in the estrogen receptor α (ERα) positive MCF-7 cells. Results showed that this induction is mediated by ERα because i) E2 did not induce HSD17B8 expression in ERα negative HepG2 cells, ii) ectopic expression of ERα restored E2-induced HSD17B8 expression, and iii) this induction was blocked by the anti-ER ICI 182 780. Additional experiments showed that no estrogen response element was necessary for this regulation. However, the CCAAT boxes located at the HSD17B8 proximal promoter were required for E2-induced transcription. Furthermore, co-immunoprecipitation studies revealed tethering of ERαto C/EBPβ in response to E2 in cells expressing ERα. Additionally, chromatin immunoprecipitation assays demonstrated that, in response to E2, ERα is recruited to the CCAAT boxes in which C/EBPβ is already bound. Taken together, our results reveal that ERα is involved in the transcriptional regulation of *HSD17B8* gene in response to E2 through its interaction with C/EBPβ.

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Introduction

The hydroxysteroid (17-beta) dehydrogenase (HSD17B) enzymes catalyze the oxidoreduction of hydroxyl/keto groups of androgens and estrogens and regulate intracellular availability of steroid hormones (Mindnich *et al.* 2004). To date, at least 14 different isozymes have been identified (Lukacik *et al.* 2006). The type 8 17β-HSD was initially characterized as a gene whose expression was down-regulated in recessive polycystic kidney disease (PKD) in the mouse (Aziz *et al.* 1993). Human and mouse HSD17B8 proteins efficiently convert estradiol (E2) into estrone (E1; Fomitcheva *et al.* 1998, Ohno *et al.* 2007). They also convert testosterone, 5α-dihydrotestosterone, and 5-androstene3β, 17β-diol into the corresponding 17-ketosteroid, but not with the same efficiency of E2. Despite its *in vitro* activity, structural studies suggested that the human protein could be involved in the metabolism of fatty acids (Pletnev & Duax 2005).

Expression of human HSD17B8 was found to be especially abundant in the prostate, placenta, and kidney (Ohno *et al.* 2007), being also detected in other tissues such as the breast, small intestine, stomach, liver, ovary, testis, and adrenals. Expression of mouse *HSD17B8* gene has been detected in several somatic tissues (Fomitcheva *et al.* 1998, Woo *et al.* 2001, Pelletier *et al.* 2005), being particularly high in the kidney and liver.

It has been described that HSD17B8 expression is regulated by estrogens in uterus and kidney (Jelinsky *et al.* 2003, Khalyfa *et al.* 2003, Bourdeau *et al.* 2004). Estrogens modulate transcription in their target tissues through estrogen receptor α (ERα) or ERβ receptors using a number of signaling pathways. The 'classical' pathway involves direct DNA binding of the activated receptor to an estrogen response element (ERE) in the promoter region of responsive genes (Misiti *et al.* 2000). The alternative, 'non-classical' pathway involves the indirect modulation of transcription by the interaction of the ER with components of other transcription complexes like FOS, SP-1, and NFκB, via protein–protein interactions (Stein & Yang 1995, Wang *et al.* 1999, DeNardo *et al.* 2005, Dong *et al.* 2006, Itoh *et al.* 2007). In addition, a 'non-genomic' pathway has been described in which estrogens can signal through membrane receptors involving MAP kinase signaling (Migliaccio *et al.* 1996).

We have previously shown that the *HSD17B8* gene is transcriptionally regulated by C/EBPβ (Villar *et al.* 2007*b*).

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Moreover, two CCAAT boxes located at −5 and −46 bp of the transcription start site were required for efficient transcription of the gene in HepG2 cells. Specific binding of C/EBPβ to these elements was detected and ectopic expression of C/EBPβ transactivated the HSD17B8 promoter. Here, we show that HSD17B8 expression is regulated by ERα in response to E2 in HepG2 cells. The HSD17B8 expression and promoter activity were induced upon E2 stimulation in cells transfected with ERα but not in untransfected cells, which demonstrates that this induction is ERα dependent. Additional experiments showed that no ERE was necessary for E2 response. However, the mutation of either of the two CCAAT boxes impaired the E2-induced HSD17B8 promoter activity, indicating that these motifs are essential for this regulation. Immunoprecipitation and ChIP assays demonstrated that ERα regulates HSD17B8 gene in response to E2 through its interaction with C/EBPβ. Taken together, our findings demonstrate that C/EBPβ and ERα participate in E2-induced HSD17B8 expression.

Materials and Methods

Reagents

HepG2 and MCF-7 cells were purchased from American Type Culture Collection (Manassas, VA, USA). Cells were cultured at 37 °C in 5% CO_2 in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal bovine serum, penicillin, and streptomycin. ERα (pHEO) expression vector was provided by Dr Pierre Chambon (Institut de Genitique et de Biologie Moleculaire et Cellulaire, Illkirch-Strasbourg, France; Green *et al.* 1986). C/EBPβ (pMSV-C/EBPβ) expression vector was a gift from Dr Steven L McKnight (UT-Southwestern Medical Center, Dallas, TX, USA; Cao *et al.* 1991). Luciferase reporter plasmids were previously described (Villar *et al.* 2007*b*). E2 and 25OH-cholesterol were supplied by Sigma–Aldrich. C/EBPβ (C-19) and ERα (HC-20) antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). ICI 182 780 (Fulvestrant) was purchased from Sigma–Aldrich.

Quantitative RT-PCR

Total RNA was isolated from HepG2 cells using the RNeasy Mini Kit (Qiagen). RNA was reverse transcribed using the Superscript II kit (Invitrogen). Real-time PCR was performed with Brilliant SYBR Green Master Mix (Stratagene, La Jolla, CA, USA) and a Chromo-4 thermocycler (MJ Research, Bio-Rad). Primers used for detecting *HSD17B8* mRNA were: sense strand 5′-ACATCAGTAGCATCG-TAGG-3′ and antisense strand 5′-GGAGGACAGAGTTA-CAGC-3′. Primers used for detecting GAPDH were: sense strand 5′-GGAGTCCACTGGCGTCTTC-3′ and antisense strand 5′-ATCTTGAGGCTGTTGTCATACTTC-3′. Results from five independent experiments, each one performed in triplicate were normalized to the level of GAPDH mRNA expressed as fold change from controls.

Transfection

Transient transfections were carried out as previously described (Villar *et al.* 2007*b*). Briefly, 5×10^5 cells were seeded in six-well plates, in triplicate, and transfected with 2 μg HSD17B8 promoter construct plus 3 ng pRL-SV40 vector as a control for transfection efficiency. Additionally, 2 μg ERα expression vector were co-transfected when indicated. Cells were harvested 24 h later, and reporter gene activity was measured in cells extracts. Luciferase activity was determined using a Berthold Lumat LB 9501 luminometer (Berthold Technologies, Oak Ridge, TN, USA) and the dual-luciferase reporter system (Promega Corp). Data were normalized to Renilla luciferase activity and total protein concentration. pGL3 basic and pGL3 control vectors (Promega) were used as transfection controls.

Immunoprecipitation and immunoblotting

After treatments, HepG2 cells were washed with PBS 1×, trypsinized and lysed with Modified-RIPA buffer (Complete Mini-EDTA protease inhibitor tablets (Roche), 0·5 M Tris–HCl pH 8·0, 10% NP-40, 1·5 M NaCl and H_2O). Five hundred micrograms of protein mixtures were then precleared with 50 μl TrueBlot anti-Rabbit IP Beads (eBioscience, San Diego, CA, USA) and incubated on ice for 1 h. Supernatants were incubated with anti-ERα, anti-C/EBPβ, or with a non-specific IgG control overnight at 4 °C, and were further incubated for another 4 h after the addition of 50 μl protein beads. After centrifugation, beads were washed at least five times with 500 μl lysis buffer, and eluted with 30 μl Laemmli Sample Buffer (Bio-Rad) with 2% β-mercaptoethanol.

Methods for SDS-PAGE electrophoresis of cellular proteins, and transfer onto polyvinylidene difluoride membranes were as previously described (Alonso *et al.* 2003, Villar *et al.* 2007*a*). Membranes were incubated with anti-ERα or anti- C/EBPβ; washed with Tween 20 in PBS; incubated with peroxidase-conjugated secondary antibody; and the signal was then detected with a chemiluminescence-based system (Pierce, Rockford, IL, USA).

Chromatin immunoprecipitation (ChIP) assay

ChIPs were performed using the ChIP Kit and Shearing Kit (Active Motif, Carlsbad, CA, USA). HepG2 cells were fixed with 37% formaldehyde and lysed according to the manufacturer's instructions. Chromatin was sheared by sonication on ice, and agarose gel electrophoresis was used to select chromatin fragments with an average size of ≤500 bp. Primers 5′-CCTAAGCAGCAGTGTCGG-3′ (upper strand) and 5′-TGTGGGTGGGTGGGAATC-3′ (lower strand) were used for PCR amplification of input DNA and DNA precipitated with anti-C/EBPβ, anti-ERα, or with a non-specific control IgG. Primers were designed to amplify a 167-bp

fragment of the HSD17B8 proximal promoter (positions −142 to +25) that encompasses the CCAAT boxes required for promoter activity.

Statistical analysis

Data were analyzed using one-factor ANOVAs. Following significance in the ANOVAs, data were subjected to analysis with Tanhane posttests in which the value necessary for significance ($P<0{\cdot}05$) is lowered by dividing the number of comparisons that are made. Analysis was done using SPSS 12.0 for Windows (Chicago, IL, USA). Results are expressed as mean ± S.E.M.

Results

E2 increases the expression of HSD17B8 in an ERα-dependent manner

Microarray studies in uterus and kidney have shown that HSD17B8 could be regulated by estrogens in those tissues (Jelinsky *et al.* 2003, Khalyfa *et al.* 2003). To confirm whether or not this gene is regulated by estrogens, we treated the breast cancer cell line MCF-7 with E2 10^{-8} M for 24 h. Real time RT-PCR showed that E2 treatment induced HSD17B8 expression by about 2·5-fold (Fig. 1A). Importantly, the anti-ER ICI 182 780 suppressed this induction, which suggested that this process is ER-dependent. To confirm this, HepG2 cells, a hepatocarcinoma cell line that does not express ERα, were treated with E2. As expected, E2 treatment does not increase HSD17B8 expression (Fig. 1B). However, when HepG2 cells were transfected with an ERα expression vector, E2 induced HSD17B8 expression by about 2·5-fold. More importantly, the anti-ER ICI 182 780 suppressed this induction which confirms that E2-induced HSD17B8 expression is dependent on ERα. Taken together, these results clearly demonstrate that HSD17B8 expression is induced by E2 by a mechanism mediated by ERα.

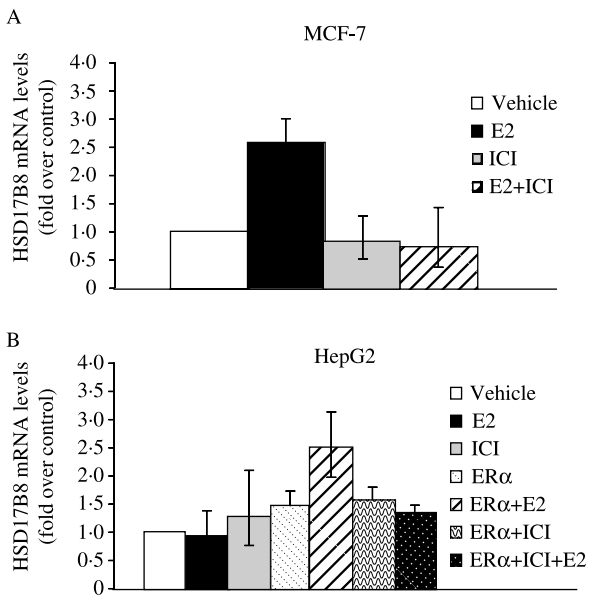

Figure 1 Estrogens activate HSD17B8 expression. (A) Effect of E2 on HSD17B8 expression in MCF-7 cells. Cells were treated with 10^{-8} M E2 and 10^{-7} M ICI 182 780 alone or in combination for 24 h. (B) Effect of E2 on HSD17B8 expression in HepG2 cells. Cells were treated with 10^{-8} M E2 and 10^{-7} M ICI alone or in combination for 24 h, in the presence or absence of ERα. Afterwards, RNA was extracted and *HSD17B8* expression measured by qPCR. GAPDH was used as loading control. Columns, mean of five independent experiments each done in triplicate; bars, S.E.M., $*P<0{\cdot}05$, in all cases.

E2 increases the expression of HSD17B8 in an ERE-independent mechanism

To further investigate the E2 activation of HSD17B8 transcription, we tested whether the promoter region of the *HSD17B8* gene responded to E2. HepG2 cells were transfected with pJV260, a construct that contained the most active part of the HSD17B8 promoter, and treated with E2 10^{-8} M for 24 h. As shown in Fig. 2, E2 treatment increased the HSD17B8 promoter activity more than 2–fold. Once again, this induction was only observed in the cells expressing ERα, which confirmed that E2-induced HSD17B8 expression is mediated by ERα. pGL3-promoter that was used as negative control and pERE, a plasmid carrying three copies of the vitellogenin ERE cloned upstream of the luciferase gene into pGL3-promoter, was used as positive control. Taken together, these data confirm that HSD17B8 expression is regulated by E2 and this is mediated by ERα.

Computational analysis on the human HSD17B8 promoter revealed the presence of a putative ERE half-site located at −82 (Villar *et al.* 2007*b*). ERα is able to activate transcription through ERE half-sites either binding directly to DNA (Kato *et al.* 1992, Ediger *et al.* 2002) or binding to other transcription factors previously bound to this element (Stossi *et al.* 2006). To evaluate whether or not this ERE half-site participates in E2-induced HSD17B8 expression, HepG2 cells were co-transfected with ERα and pJV260 pJV150, or pJV75. pJV260 and pJV150 constructs contained the ERE half-site. However, pJV75 is a deletion construct lacking this element. As shown in Fig. 2, treatment with E2 induced the promoter activity of all of them by more than 2–fold, which demonstrates that this element is not implicated in the regulation of HSD17B8 expression and suggests that *HSD17B8* gene is transcriptionally regulated by E2 in an ERE-independent mechanism.

E2 action on HSD17B8 is through the CCAAT boxes presented at the promoter of the gene

Previous studies demonstrated that two CCAAT boxes located at −5 and −46 are essential for HSD17B8 promoter activity (Villar *et al.* 2007*b*). Furthermore, C/EBPβ bound to these boxes *in vitro* and transactivated the HSD17B8

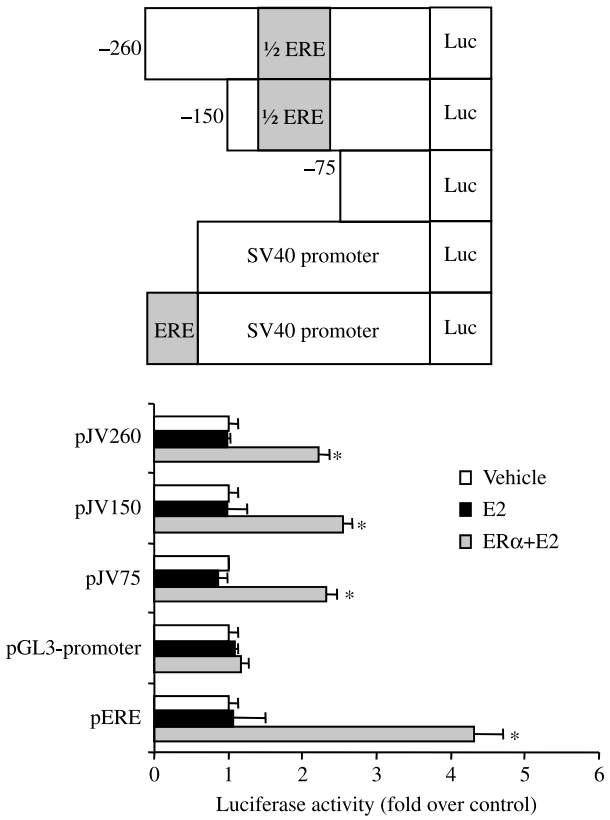

Figure 2 E2 increases the HSD17B8 promoter activity in an ERE-independent manner. HepG2 cells were transfected with various human HSD17B8 promoter deletion constructs (schematic on the left) in the presence or absence of E2 10^{-8} M and ERα. Promoter activity (right) was determined as described in Materials and Methods and expressed as -fold over control (cells treated with vehicle). Columns, mean of five independent experiments each done in triplicate; bars, S.E.M., *$P<0{\cdot}05$, in all cases.

promoter activity. After showing that E2 induces the transcriptional activity of the minimal promoter, it became important to explore whether or not these CCAAT boxes participate in E2-induced HSD17B8 promoter activity. Therefore, HepG2 cells were transfected with pJV260 or with constructs in which these CCAAT boxes are mutated. E2 treatment increased the promoter activity of the wild-type pJV260 by about 2·5-fold. However, mutation of either of the two CCAAT boxes prevented the E2-induced HSD17B8 promoter activity (Fig. 3). These results demonstrate that CCAAT boxes located at −5 and −46 relative to the start site are necessary for E2-induced HSD17B8 transcription.

Estrogens do not regulate C/EBPβ expression in HepG2 cells

Estrogens are known to regulate expression of some transcription factors like C-MYC (Dubik *et al.* 1987), FOS, Sp1, and NF-YA (Dong *et al.* 2007). Recently, it has been described that dehydroepiandrosterone modulates the transcription of HSD11B2 by increasing C/EBPβ expression

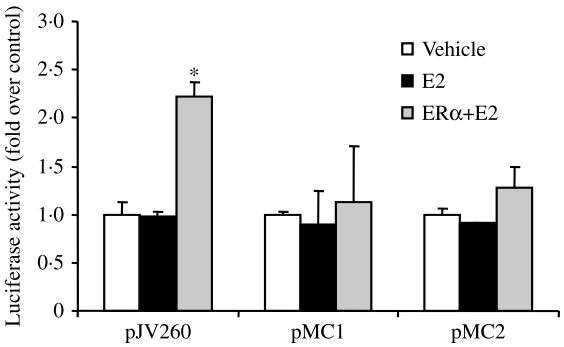

Figure 3 CCAAT boxes are essential for the E2-induced HSD17B8 expression. CCAAT boxes located at −5 (pMC1) and −46 (pMC2) were mutated. HepG2 cells were co-transfected with the different mutants and the ERα expression vector, and treated with 10^{-8} M E2. Promoter activity was determined as previously described and expressed as -fold over control (cells treated with vehicle). Columns, mean of five independent experiments each done in triplicate; bars, S.E.M., *$P<0{\cdot}05$, in all cases.

(Balazs *et al.* 2007). Since C/EBPβ transactivates HSD17B8, upregulation of C/EBPβ by E2 would explain the induction of HSD17B8 expression by E2. To check if E2 increases C/EBPβ expression, MCF-7 and HepG2 cells expressing ERα were treated with E2 10^{-8} M. Real time RT-PCR showed that E2 does not induce HSD17B8 expression in either of the cell lines (Fig. 4), which clearly indicates that E2-induced HSD17B8 expression is not mediated by the upregulation of C/EBPβ.

C/EBPβ and ERα interact in HepG2 cells expressing ERα

A direct interaction between ERα and C/EBPβ bound to DNA has been described in MCF-7 cells (Dong *et al.* 2006). Since C/EBPβ transactivates HSD17B8 (Villar *et al.* 2007*b*) and E2-induced HSD17B8 expression is ERE-independent, we then decided to test whether or not ERα interacts with C/EBPβ to promote HSD17B8 expression in response to E2. Therefore, HepG2 cells expressing ERα were treated with E2 and co-immunoprecipitation assays with C/EBPβ (Fig. 5A) or ERα (Fig. 5B) specific antibodies were performed. Immunoprecipitated complexes were detected with either ERα (Fig. 5A) or C/EBPβ (Fig. 5B). IgG was included as negative control. In the absence of E2, ERα showed minimal interaction with C/EBPβ (Fig. 5A and B, middle panels). However, upon E2 treatment there was an increase in the amount of the complex formed between ERα and C/EBPβ (Fig. 5A and B, bottom panels). No interaction was observed in cells not expressing ERα (Fig. 5A and B, top panels). Taken together these results indicate that E2 promotes the formation of a complex between ERα and C/EBPβ.

Recruitment of ERα to HSD17B8 promoter in response to E2

After showing that E2 induces the formation of the complex ERα-C/EBPβ, we next investigated the recruitment of ERα

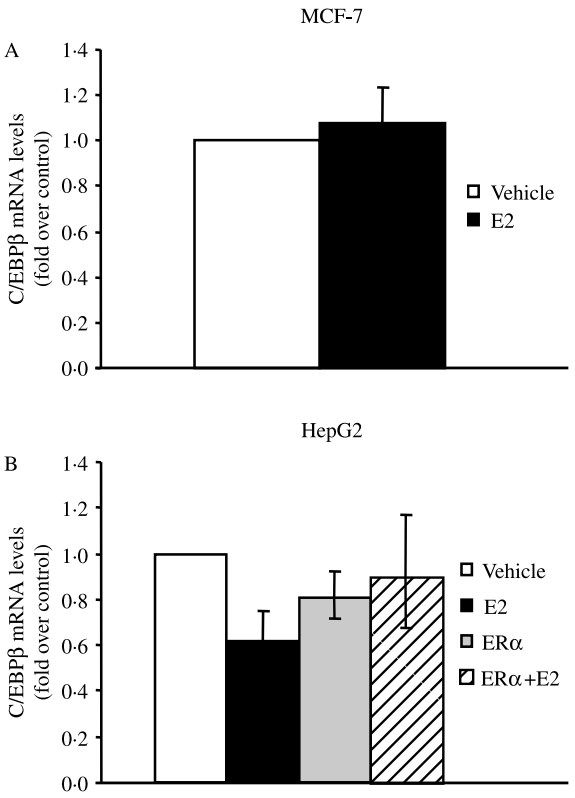

Figure 4 E2 does not regulate C/EBPβ expression. (A) MCF-7 cells were treated with 10^{-8} M E2 for 24 h. (B) HepG2 cells were treated with 10^{-8} M E2 for 24 h in the presence or absence of ERα. Then, RNA was extracted and *C/EBPβ* expression was measured by qPCR. GAPDH was used as loading control. Columns, mean of five independent experiments each done in triplicate; bars, S.E.M., $^{*}P<0{\cdot}05$, in all cases.

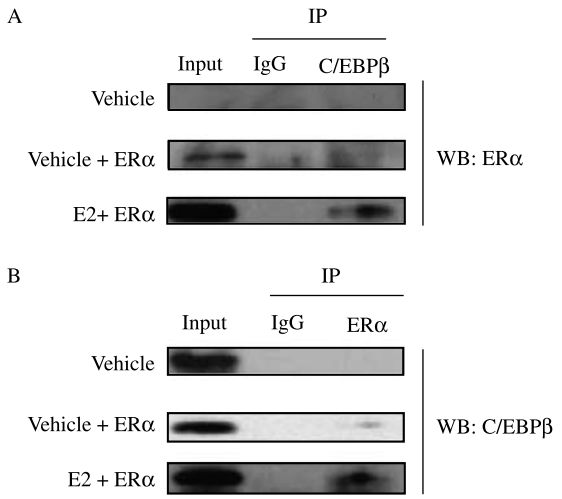

Figure 5 E2 induces interaction between C/EBPβ and ERα. HepG2 cells were treated with 10^{-8} M E2 or vehicle for 24 h in the presence or absence of ERα. After treatments, cells were harvested and protein complexes immunoprecipitated (IP) using specific antibodies against C/EBPβ, ERα, or control IgG. Western blots (WB) with (A) C/EBPβ or (B) ERα specific antibodies were performed as described in Materials and Methods.

Discussion

HSD17B8 protein efficiently converts E2 into E1 *in vitro*, which suggests that it might be involved in the metabolism of estrogens. Here, we show that HSD17B8 is regulated by estrogens (Figs 1 and 2), and that this regulation is ERα-mediated. Results from MCF-7 cells (Fig. 1A), a breast cancer

and C/EBPβ to the HSD17B8 promoter in response to E2. Therefore, ChIP assays were performed in HepG2 cells expressing ERα treated with 10^{-8} M E2 or vehicle. HepG2 cells not expressing ERα exposed to vehicle were used as a control. Chromatin was immunoprecipitated with ERα, C/EBPβ, or IgG specific antibodies. For PCR analysis a set of primers that amplified a fragment that encompassed the CCAAT boxes located within the proximal promoter of HSD17B8 were used (Fig. 6A). As expected, no ERα recruitment was observed in cells not expressing ERα (Fig. 6B, top panel). Interestingly, in the absence of hormone, ERα was slightly recruited to the HSD17B8 promoter (6B, middle panel). However, after E2 treatment, the recruitment of ERα to the HSD17B8 promoter was highly induced (6B, lower panel). On the other side, recruitment of C/EBPβ to the HSD17B8 promoter was not affected by E2 treatment nor ERα expression, which suggests that C/EBPβ is not affected by E2 treatment. Based on these findings, we conclude that in response to E2, ERα binds to C/EBPβ already bound to HSD17B8 5′ untranslated region promoting its expression.

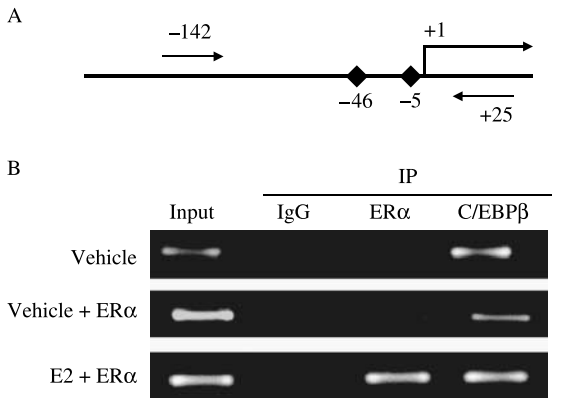

Figure 6 Recruitment of ERα to the HSD17B8 promoter in response to E2. (A) Panel illustrating the position of the HSD17B8 promoter primers used for the amplification of the ERα, C/EBPβ, or IgG immunoprecipitated DNA fragments. (B) Chromatin immunoprecipitation (ChIP) assay showing that ERα bound to the 17HSD8 promoter upon E2 (10^{-8} M) stimulation for 24 h. ChIP was performed with chromatin prepared from untransfected HepG2 cells treated with vehicle (top panel), transfected with ERα HepG2 cells treated with vehicle (middle panel) and transfected with ERα HepG2 cells treated with 10^{-8} M E2. Results of amplification of soluble chromatin before precipitation are shown as control (input).

cell line ERα positive, and from HepG2 cells (Fig. 1B), a hepatocarcinoma cell line with no ERα expression, revealed that in the presence of ERα E2-induced HSD17B8 expression by about 2·5-fold. This induction was blocked by the addition of the anti-estrogens ICI 182 780, and no induction was observed in the absence of ERα in HepG2 cells, which clearly demonstrates that E2-induced HSD17B8 expression is through ERα.

Our results confirm previous results that showed that the *HSD17B8* gene is regulated by estrogens in uterus and kidney (Jelinsky *et al.* 2003, Khalyfa *et al.* 2003, Bourdeau *et al.* 2004). The biological effects of estrogens are mediated by ERα and ERβ, which are members of the superfamily of nuclear receptors (Bjornstrom & Sjoberg 2005). The classical mechanism of ER action involves estrogens binding to receptors in the nucleus, after which the receptors dimerize and bind to specific response elements (ERE) in the promoter of target genes. Although, Bourdeau *et al.* identified a putative ERE about 3 kb upstream of the transcription start site of the human HSD17B8, we demonstrate that transcriptional regulation of HSD17B8 by ERα does not require direct binding of the agonist-activated ERα to this element (Fig. 2). The human HSD17B8 proximal promoter contains an ERE half-site located at −82. It has been described that ERE half-sites bind ERα but not ERβ (Vanacker *et al.* 1999). Our results clearly show that not the ERE half-site but CCAAT boxes present in the human HSD17B8 proximal promoter are required for the induction of HSD17B8 expression by E2 (Fig. 3).

In addition to the classical mechanism, two other different mechanisms called non-genomic and non-classical have been described for ER action. The non-classical mechanism, often referred as transcriptional crosstalk, involves tethering of the receptor with other transcription factor complexes that contact the DNA. In previous work, we demonstrated that CCAAT boxes located at −5 and −46 contribute dramatically to the basal promoter activity of HSD17B8. We also detected specific binding of C/EBPβ to those motives, and we showed that ectopic expression of C/EBPβ transactivated the HSD17B8 promoter. Here, we describe that C/EBPβ and ERα crosstalk to promote HSD17B8 transcription in response to E2 (Figs 5 and 6). Interestingly, previous studies have shown C/EBPβ and ERα transcriptional crosstalk, to repress the human IL6 promoter in human osteoblast cell lines (Stein & Yang 1995) and to activate the human prolactin receptor gene in human breast cancer MCF-7 cells (Dong *et al.* 2006). In the latter, E2 induced the formation of a complex between C/EBPβ, Sp1, and ERα. In the present study, using co-IP, we provide strong evidence of an interaction between ERα and C/EBPβ in response to E2 in human hepatoma HepG2 cells (Fig. 5). Our results also show that E2 induces recruitment of ERα but not C/EBPβ to the HSD17B8 promoter (Fig. 6). This suggests that E2-activated ERα tethers to C/EBPβ already bound to the HSD17B8 promoter, thus, enhancing its expression.

C/EBPβ belongs to a family of transcription factors that contain a highly conserved, basic leucine zipper domain at the C-terminus that is involved in dimerization and DNA binding (Ramji & Foka 2002). C/EBPβ is a critical regulator of proliferation and/or differentiation in multiple tissues, including the liver, adipose tissue, immune system, and mammary gland (Wedel & Ziegler-Heitbrock 1995, Greenbaum *et al.* 1998, Seagroves *et al.* 1998, Tang *et al.* 2003). Importantly, C/EBPβ is essential for female reproduction because of a critical role in ovarian follicle development (Sterneck *et al.* 1997). C/EBPβ is known to be a critical mediator of steroid hormone responsiveness in the uterus (Mantena *et al.* 2006), where it mediates E2-induced epithelium proliferation and, additionally, is rapidly induced by E2. In the present study, we clearly show that the induction of HSD17B8 expression by E2 in HepG2 cells is not due to an increase in C/EBPβ expression after E2 treatment (Fig. 4).

C/EBPβs transcription factors have been involved in the regulation of HSD11B1 and 11β-HSD2, enzymes involved in glucocorticoid metabolism (Williams *et al.* 2000, Gout *et al.* 2006). Impaired intracellular metabolism of steroids has been suggested to contribute to the development of various pathologic conditions including PKD. Interestingly, HSD17B8 and HSD11B1 expression are downregulated in PKD (Aziz *et al.* 1994), which suggests that C/EBPβs transcription factor might play an important role in PKD. A significant decrease in HSD17B8 expression has also been described in tumor tissue of oral cavity patients without lymph node metastasis compared with its surrounding healthy tissue (Reinders *et al.* 2007). Whether or not ERα and C/EBPβ play a role in this downregulation remains to be investigated.

In conclusion, the fact that HSD17B8 converts E2 into E1 suggests that HSD17B8 plays an important role in the control of the intracellular levels of active estrogens. Here, we show that estrogens regulate the expression of *HSD17B8*, a gene implicated in their own regulation. We also provide strong evidence of C/EBPβ role in E2/ERα mediated HSD17B8 expression. However, the molecular mechanisms underlying the effects of estrogens are likely to be specific for the cell type, and thus, the gene responses are likely to be diverse. Therefore, additional experiments are required to demonstrate if HSD17B8 is regulated by E2 in tissues where estrogens play an important role in the initiation and progression of cancer.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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