Biochemical and Biophysical Research Communications 500 (2018) 275–282

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Sterol regulatory element binding protein 1 trans-activates 25-hydroxy vitamin D_3 24-hydroxylase gene expression in renal proximal tubular cells

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ARTICLE INFO

Article history:
Received 2 April 2018
Accepted 9 April 2018
Available online 17 April 2018

Keywords:
Vitamin D
CYP24A1
SREBP1
Thyroid hormone
Transcriptional regulation

ABSTRACT

The physiological activity of the steroid derived hormone vitamin D is regulated by several enzymatic steps. Both 25-hydroxy vitamin D_3 1α-hydroxylase (CYP27B1) and 25-hydroxyvitamin D_3 24-hydroxylase (CYP24A1) modulate blood levels of 1,25-dihydroxyvitamin D_3, an activated form of vitamin D. We previously demonstrated that *CYP27B1* expression was trans-activated by sterol regulatory element binding protein 1 (SREBP1), although whether SREBP1 also regulates *CYP24A1* transcription was unclear. Here we investigated the ability of SREBP1 to affect *CYP24A1* transcription. In a luciferase reporter assay, mouse and human *CYP24A1* promoter activity was strongly activated by SREBP1 in opossum kidney proximal tubular cells (OK-P). Three putative SREs (pSREs) were found in the mouse *Cyp24a1* gene promoter and the SREBP1 protein showed specific binding to the pSRE1 element in EMSAs. Site-directed mutagenesis of the pSRE1 element strongly decreased SREBP1-mediated *Cyp24a1* gene transcription. Moreover, siRNA-mediated *SREBP1* knock-down repressed *CYP24A1* expression in human renal proximal tubular epithelial cells (HKC-8). In animal studies, mice given various doses of thyroid hormone (T_3) showed dose-dependent reductions in renal *Srebp1c* and *Cyp24a1* mRNA levels. Taken together, our results suggest that SREBP1 trans-activates *CYP24A1* expression through SREBP binding elements present in the promoter.

1. Introduction

Vitamin D regulates various biological actions such as bone metabolism, phosphate and calcium metabolism, cellular differentiation and immune systems. Most of these functions are thought to be mediated by the vitamin D receptor (VDR), which belongs to the nuclear receptor superfamily. 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the activated form of vitamin D, is produced through a two-step hydroxylation. In the liver, vitamin D is first converted to 25-hydroxyvitamin D and is subsequently modified in the kidney by 1a-hydroxylase (CYP27B1) to form 1,25-dihydroxyvitamin D_3. To avoid effects of excess $1,25(OH)_2D_3$, inactivation of vitamin D modifying systems is critical. 25-hydroxyvitamin D_3 24-hydroxylase (CYP24A1) catalyzes the synthesis of 1,24,25-trihydroxyvitamin D_3 and 24,25-dihydroxyvitamin D_3, which are inactive forms of vitamin D. *CYP24A1* is expressed in the kidney, osteoblastic cells and many other tissues to mediate systemic and local vitamin D inactivation [1,2]. The expression of both *CYP27B1* and *CYP24A1* are regulated by parathyroid hormone (PTH), $1,25(OH)_2D_3$ and fibroblast growth factor-23 (FGF-23) [3,4].

We previously revealed the involvement of thyroid hormone in

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https://doi.org/10.1016/j.bbrc.2018.04.058
0006-291X/

vitamin D metabolism. The thyroid hormone triiodothyronine (T_3) regulates 1,25$(OH)_2$D homeostasis through negative transcriptional regulation of *CYP27B1* expression in renal proximal tubular cells. Moreover, a sterol regulatory element (1α-SRE) that is known to the responsive element for SRE binding protein (SREBP) was identified in the *CYP27B1* promoter. SREBP1c and SREBP1a both activate *CYP27B1* transcription [5].

SREBPs are transcriptional factors that play an important role in lipid metabolism as master transcriptional regulators. The mammalian genome encodes three SREBP isoforms: SREBP1c, SREBP1a and SREBP2. Both SREBP1c and 1a are expressed from a single gene through the use of alternative transcription start sites that produce alternate forms of exon 1, whereas SREBP2 is encoded by a different gene. SREBP1c preferentially enhances transcription of genes required for fatty acid synthesis and SREBP2 preferentially activates cholesterol synthesis. SREBP1a activates both fatty acid and cholesterol synthesis [6,7].

Although SREBP1 is known to activate *CYP27B1* transcription [5], whether SREBP1 regulates *CYP24A1* expression is unclear. Here we investigated regulation of *CYP24A1* expression by SREBP1 and identified a novel SREBP binding element in the *CYP24A1* promoter.

2. Materials and methods

2.1. Cell culture

OK-P (opossum kidney proximal tubule), HepG2 (human hepatocellular carcinoma), HCT116 (human colorectal carcinoma) and Saos-2 (human osteosarcoma) cells were cultured in DMEM (Dulbecco's modified Eagle's medium; Life Technologies, Grand Island, NY, USA). HKC-8 (human kidney proximal tubule) cells were cultured in DMEM/Ham's F-12 (Wako, Osaka, Japan). Cell cultures were maintained at 37 °C under an atmosphere containing 5% CO_2. Growth media were supplemented with 10% or 5% fetal bovine serum (FBS) (Life Technologies, Grand Island, NY, USA), 100 units/ml penicillin, and 0.1 mg/ml streptomycin (Sigma-Aldrich Japan, Tokyo, Japan).

2.2. Plasmid construction

The luciferase reporter plasmid rat *Fasn* (Fatty acid synthase)-Luc was previously described [8]. Plasmids carrying the human and mouse *CYP24A1* promoter (ph24A-1.49k, pm24A-960, pm24A-217, pm24A-122, and pm24A-69) were constructed by PCR amplification using gene-specific primers (Table 1) and cloned into the pGL4.12-basic vector (Promega KK, Tokyo, Japan) digested with BglII. Human *SREBP1c* (amino acids 1–449) and human *SREBP1a* (amino acids 1–473) expression plasmids in a pcDNA3.1/Myc-His(+) vector were constructed as previously described [8]. The human vitamin D receptor (VDR) expression vector pSG5-VDR and the mouse retinoid X receptor (RXR) alpha expression vector pSG5-RXRα were previously described [9]. The plasmid pm24A-217 encoding mutated SRE1 was constructed using the QuikChange site-directed mutagenesis kit (Agilent Technologies, Palo Alto, USA) with the oligonucleotides shown in Table 3. Each plasmid was purified using the Pure Yield Plasmid Midiprep System (Promega KK, Tokyo, Japan).

Table 1
Oligonucleotides used in reporter plasmid construction.

Name	Sequence (5′ to 3′)
pmCyp24A-960-Bgl-S	GAAGATCTATCCAGATACAAAAGCAAG
pmCyp24A-217-Bgl-S	GAAGATCTCCGGGGTGGAGTC
pmCyp24A-122-Bgl-S	GAAGATCTTCCCACACCCGCCCCCCG
pmCyp24A-69-Bgl-S	GAAGATCTCAGCGTGCTCATTGGCCAC
pmCyp24A+211-Hind-AS	CCCAAGCTTGGAAGAGGCAGATGCCCAG
phCyp24A-1.49k-S	GGAGATCTACATACTGTATGCAATC
phCyp24A+80-Bgl-AS	GGAGATCTAGGGTCTGGCTGGAGCCAC

2.3. Transfection and luciferase reporter assays

Cell transfections were performed using the Lipofectamine 2000 reagent (Life Technologies, Grand Island, NY, USA) as previously described [5]. OK-P cells were transfected with 0.4 μg luciferase reporter plasmid and 0.2 μg expression vector. The DNA/Lipofectamine mixtures were removed after 4 h, and the cells were grown in DMEM containing 10% FBS and treated with 1,25$(OH)_2D_3$ (Solvay Pharmaceuticals, Marietta, GA, USA) or ethanol vehicle for an additional 18 h. Normalization of luciferase activity for transfection efficiency was achieved by co-transfection with 0.2 μg pCMV-β (Agilent Technologies, Palo Alto, CA, USA), a *β-gal* (β-galactosidase) expression vector. Cells were harvested in cell lysis buffer (Promega KK, Tokyo, Japan) and the lysates were assayed for luciferase activity and *β-gal* activity.

2.4. Coupled transcription/translation assays

The human SREBP1c (amino acids 1–449) and human SREBP1a (amino acids 1–473) proteins were each separately synthesized using the TNT Quick Coupled Transcription/Translation System (Promega KK, Tokyo, Japan) at 30 °C for 90 min in the presence of 20 μM methionine. The proteins were then used in electrophoretic mobility-shift assays (EMSAs).

2.5. EMSAs

EMSAs were performed as previously described [5]. Double-stranded oligonucleotides for mouse *CYP24A1*-putative SRE1 (pSRE1), pSRE2, pSRE3, pSRE1-mutant, AP-1 consensus, human *LDLR* (Low density lipoprotein receptor)-SRE and rat *Fasn*-SRE were synthesized (Table 2) and purified electrophoretically on 1% agarose gels. The purified DNA fragments were end-labeled using [γ-^{32}P] ATP (110 TBq/mmol; ICN Pharmaceuticals, Costa Mesa, CA, USA) and T4 polynucleotide kinase (Takara Bio Inc., Shiga, Japan). Gel shift assays were performed with 2 μl of the appropriate *in vitro*-translated protein. Proteins were incubated on ice for 30 min in binding buffer (20 mM HEPES-KOH pH 7.9, 1 mM EDTA pH 8.0, 50 mM KCl, 5% glycerol, 10 mM DTT, 0.5 mM PMSF) in a total volume of 20 μl before addition of labeled probes and incubation for 30 min at room temperature. Binding reaction specificity was determined using 100-fold molar excess of the relevant unlabeled competitor oligonucleotide. SREBP1 components of protein–DNA complexes were analyzed using antibodies specific for SREBP1 and c-fos (sc-8984x and sc-253x, respectively; Santa Cruz Biotechnology, CA, USA). The incubated samples were then electrophoresed on 6% (w/v) polyacrylamide gels in 0.25× TBE (22.5 mM Tris, 22.5 mM boric acid, 0.5 mM EDTA, pH 8.0) running buffer for 2 h at 150 V. The gel was dried and analyzed with a Fluorescent Image Analyzer FLA-9000 equipped with Multi-Gauge Version 3.0 software (Fujifilm, Tokyo, Japan).

2.6. RNA interference

HKC-8 cells were cultured in 35 mm culture plates at 37 °C under an atmosphere containing 5% CO_2. Upon reaching 50% confluence, cells were transfected. RNA mixtures containing control siRNA (AAGUCACGACUAGAUAUUGACUUUG, CAAAGUCAAUAUCUAGUCGUGACUU) or 100 pmol SREBP1 siRNA (AAGACAGCAGAUUUAUUCAGCUUUG, CAAAGCUGAAUAAAUCUGCUGUCUU) and

Table 2
Oligonucleotide sequences used for EMSAs.

Gene Name	Sense (5′ to 3′)	Antisense (5′ to 3′)
mouse *Cyp24a1* pSRE1	GTGTCGGTCACCCGAGGCCCC	CGGGGCCTCGGGTGACCGACA
mouse *Cyp24a1* pSRE2	CGGCGCCCTCACTCACCTCGCTGACTCCA	ATGGAGTCAGCGAGGTGAGTGAGGGCGCC
mouse *Cyp24a1* pSRE3	GGTTATCTCCGGGGTGGAGTCC	TGGACTCCACCCCGGAGATAAC
mouse *Cyp24a1* pSRE1 mutant	GTGTCGGTTGCCCGAGGCCCC	CGGGGCCTCGGGCAACCGACA
human *CYP24A1* pSRE1	GCCCCGGTCACCCCAGGCCCG	CCGGGGCCTGGGGTGACCGGGG
human *LDLR*-SRE	TTTGAAAATCACCCCACTGCAAACT	AGTTTGCAGTGGGGTGATTTTCAAA
rat *Fasn*-SRE	GCGCGGGCATCACCCCACCGACGGC	GCCGTCGGTGGGGTGATGCCCGCGC
AP1 consensus	GGCGCTTGATGACTCAGCCGGAA	GGTTCCGGCTGAGTCATCAAGCG

Table 3
Oligonucleotides used in mutagenesis.

Name	Sequence (5′ to 3′)
mouse *Cyp24a1* pSRE1 mutant-S	CCATGCCCTCAAGGTGTTCCACAGTCTCCC
mouse *Cyp24a1* pSRE1 mutant-AS	GGGAGACTGTGGAACACCTTGAGGGCATGG

5 μl Lipofectamine 2000 (Life Technologies, Grand Island, NY, USA) added to 50 μl serum-free media were prepared. Both solutions were incubated for 20 min at room temperature then the transfection mixtures were added to each plate containing 1.5 ml serum-free media. The siRNA/Lipofectamine mixture was removed after 4 h, and the cells were then grown for 24 h in culture medium. After incubation, the cells were harvested with RNAiso plus regent (Takara Bio Inc., Shiga, Japan).

2.7. RT-PCR and real-time PCR analysis

Extraction of total RNA, cDNA synthesis, RT-PCR and real-time PCR analysis were performed as previously described [9,10]. Briefly, 2 μl cDNA was used for RT-PCR with GoTaq Green Master Mix (Promega KK, Tokyo, Japan). The amplified PCR products were then separated on 2% agarose gels. Real-time PCR analysis was performed using the indicated primers (Table 4) in a Light Cycler (Roche Diagnostics, Mannheim, Germany) and the StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The amplification products were analyzed using a melting curve, which confirmed the presence of a single PCR product in all reactions (except for negative controls). The PCR products were quantified by fit-point analysis, and results were normalized to those for *β-actin*.

2.8. Experimental animals

Seven-week-old C57BL/6 male mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). The animals were housed in pathogen-free conditions and maintained under a standard 12-h light-dark cycle with free access to water and standard diet ad libitum. The experimental groups were given T_3 (Sigma-Aldrich Japan, Tokyo, Japan) by i.p. injection at 1, 5 and 20 μg/100 g body weight (n = 5 per group) [5]. The animals in the control group were given a similar volume of saline (sham-injected controls). At 24 h after injection, all animals were sacrificed under pentobarbital anesthesia. The kidneys were rapidly removed and stored at −80 °C until analysis. All experiments involving animals were conducted in accordance with the Guidelines for Animal Experimentation of the Tokushima University School of Medicine.

2.9. Statistical analysis

Data are expressed as means ± SD. Statistical significance was determined by Student's unpaired *t*-test or one-way ANOVA followed by the Dunnett's post hoc test. p-values ($p < 0.05$) were considered significant.

3. Results

3.1. Transcriptional regulation of the CYP24A1 promoter by SREBP1

To determine whether SREBP1 up-regulates *CYP24A1* promoter activity, we first constructed reporter plasmids containing mouse- or human-derived *CYP24A1* for use in luciferase reporter assays. Both SREBP1c and SREBP1a trans-activated in each luciferase reporter plasmid and each SREBP1 isoform also induced trans-activation of Fasn-Luc, a typical SREBP1 target gene (Fig. 1A–C). In the presence of $1,25(OH)_2D_3$, SREBP1c additively activated pm24A-960 (Fig. 1D).

3.2. Identification of a putative sterol regulatory element (pSRE) in the mouse Cyp24a1 gene promoter

To identify SREBP1-responsive-elements in the *CYP24A1* promoter, multiple luciferase reporters with deletions in the promoter region were constructed (pm24A-960, pm24A-217, pm24A-122 and pm24A-69). SREBP1c trans-induction was remarkably decreased in constructs that had promoter lengths shorter than −217 (Fig. 2A). We noted three putative SRE (pSRE) sequences, pSRE1 (5′-TCACCCGAG-3': −186 to −178), pSRE2 (5′-TCACCTCGCT-3': −161 to −153) and pSRE3 (5′-TCCGGGGTG-3': −236 to −228) in the vicinity of the proximal promoter region. Each of these pSRE

Table 4
Oligonucleotides used for real-time PCR.

Gene Name	Sense (5′ to 3′)	Antisense (5′ to 3′)
mouse *β-actin*	CTGACCCTGAAGTACCCCATTGAACA	CTGGGGTGTTGAAGGTCTCAAACATG
mouse *Cyp24a1*	TGCCATTCACAACTCGGACCC	TCAAGCCAGCGTTCGGGTCTAA
mouse *Srebp1c*	ATCGGCGCGGAAGCTGTCGGGGTAGCGTC	ACTGTCTTGGTTGTTGATGAGCTGGAGCAT
mouse *Srebp1a*	GCGCCATGGACGAGCTG	TTGGCACCTGGGCTGCT
human *β-ACTIN*	GGCACCACACCTTCTACAATGAGC	AGCCTGGATAGCAACGTACATGGC
human *CYP24A1*	GCCGTATTTAAAAGCCTGTCTGAA	ACCTGGGTATTTAGCATGAGCACTG
human *SREBP1c*	GGAGCCATGGATTGCACTTT	ATGTGGCAGGAGGTGGAGAC
human *SREBP1a*	GCTGCTGACCGACATCGAA	ATGTGGCAGGAGGTGGAGAC

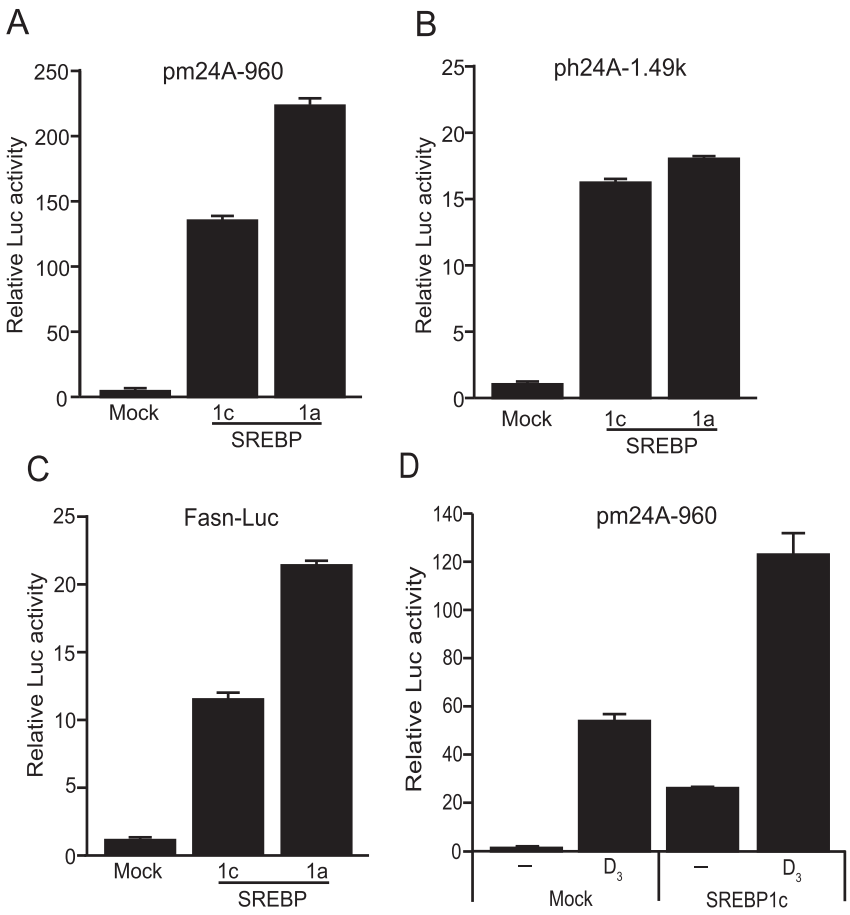


Fig. 1. SREBP1 activates *CYP24A1* transcription. A-C) OK-P cells were co-transfected with mouse *Cyp24a1* promoter −960b (pm24A-960), human *CYP24A1* promoter −1.49k (ph24A-1.49k) or rat *Fasn* promoter −444b (*Fasn*-Luc) and pCMV-β, pcDNA3.1-*SREBP1c*, pcDNA3.1-*SREBP1a* or empty vector. Cells were incubated for 18 h prior to lysis and measurement of *β-gal* and luciferase activity. D) OK-P cells were co-transfected with pm24A-960 and pCMV-β, pSG5-VDR, pSG5-RXRα, pcDNA3.1-*SREBP1c* or empty vector. Cells were incubated for 18 h with or without 10^{-8} M $1,25(OH)_2D_3$. Cell lysates were assayed for *β-gal* and luciferase activity. All data are represented as means ± SD (n = 2). Similar results were obtained from two or more independent experiments.

sequences was consistent with both mouse and human sequences (Fig. 2B).

3.3. Importance of pSRE for SREBP1 binding specificity and transcriptional induction

To show the specific binding of SREBP1 to the *CYP24A1* promoter, gel-shift assays were next performed. Three oligonucleotides containing one of the mouse-derived pSREs were prepared. Only pSRE1 showed band shifting (Fig. 3A). SREBP1a also showed band shifting with pSRE1 (Fig. 3B). To confirm the binding specificity of SREBP1c for the mouse *Cyp24a1* and human CYP24A1 oligonucleotides, we also used several competitors (two consensus SRE probes; human *LDLR*, rat *Fasn*, pSRE1 mutant oligonucleotides, AP-1 consensus). These consensus SRE competitors inhibited SREBP1c binding, whereas the pSRE1 mutant oligonucleotide and AP-1 consensus had no effect. Binding specificity was confirmed to be similar using a specific antibody (Fig. 3C and D). A reporter plasmid carrying a mutation in pSRE1 inhibited trans-induction by SREBP1c to levels that were less than one-fifth of the wild type (Fig. 3E).

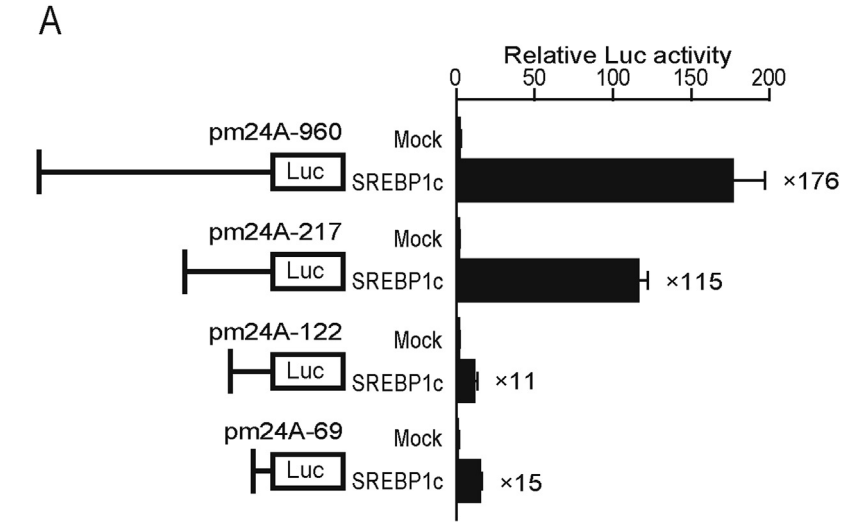
3.4. Effect of decreased SREBP1 expression on CYP24A1 mRNA levels in vitro and in vivo

RT-PCR of endogenous mRNA expression of *SREBP1c*, *SREBP1a* and *CYP24A1* in HKC8 cells showed mRNA levels that were detectable and similar to that seen for other human-derived cell lines (Fig. 4A). Compared with the control group, the group transfected with *SREBP1*-specific siRNA had significantly reduced mRNA expression of each *SREBP1* isoform and *CYP24A1* (Fig. 4B).

We next examined SREBP1-mediated regulation of *CYP24A1* expression *in vivo*. Renal mRNA levels of *Srebp1c* and *Srebp1a* were measured in mice injected with increasing amounts of T_3, which was previously shown to negatively inhibit *Cyp27b1* transcription that in turn affects $1,25(OH)_2D$ homeostasis [5]. T_3 treatment dose-dependently decreased *Srebp1c* mRNA levels (Fig. 4C), whereas *Srebp1a* mRNA levels were not significantly affected by T_3 treatment (Fig. 4D). *Cyp24a1* mRNA levels decreased in the same manner as *Srebp1c* (Fig. 4E).

4. Discussion

In the current study, we demonstrated that both SREBP1 isoforms trans-activate *CYP24A1* via a SRE element. A previous study demonstrated that hepatic *Srebp1c* mRNA levels were decreased in mice injected with T_3 [11]. Furthermore, *Srebp1c* mRNA levels were down-regulated in T_3-treated human primary adipose tissues [12]. Here we showed that renal *Srebp1c* mRNA levels were specifically and dose-dependently down-regulated in T_3-treated mice (Fig. 4C). Although the physiological role of decreases in renal *Srebp1c* levels is unclear, our finding that *CYP24A1* mRNA levels were repressed



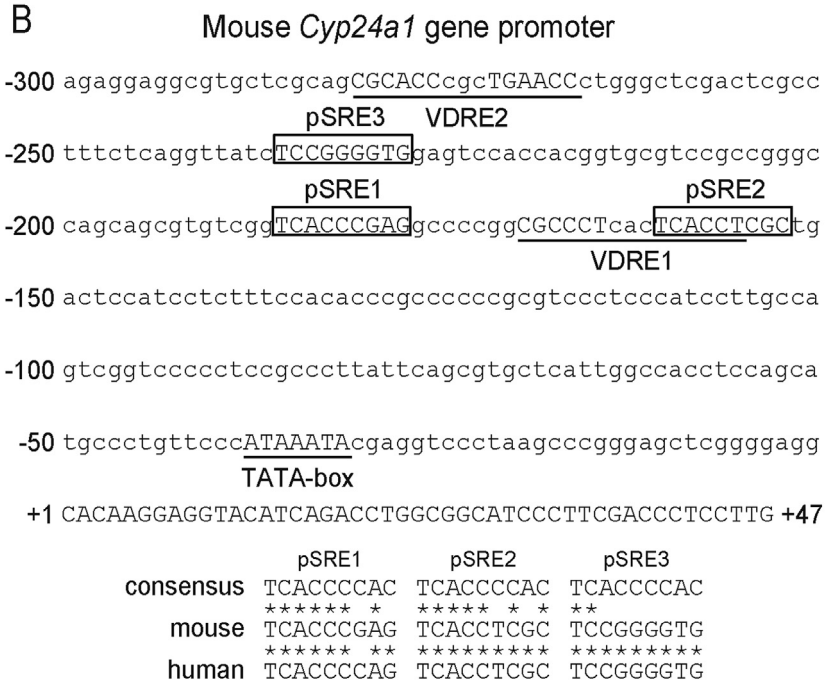


Fig. 2. Identification of a putative SRE in the *CYP24A1* promoter. A) OK-P cells were co-transfected with the indicated reporter constructs and pCMV-β, pcDNA3.1-*SREBP1c* or empty vector. Luciferase activity was normalized for *β-gal* activity. All data are represented as means ± SD (n = 2). B) Sequence of the mouse *Cyp24a1* gene promoter region corresponding to −300 to +47 bp containing putative (p) SRE1 (−186 to −178), pSRE2 (−161 to −153), pSRE3 (−236 to −228), TATAbox (−38 to −32), VDRE1 (−170 to −156) and VDRE2 (−281to −267) sequences. The homology of each pSRE in the human *CYP24A1* gene promoter and consensus SRE sequence are shown below.

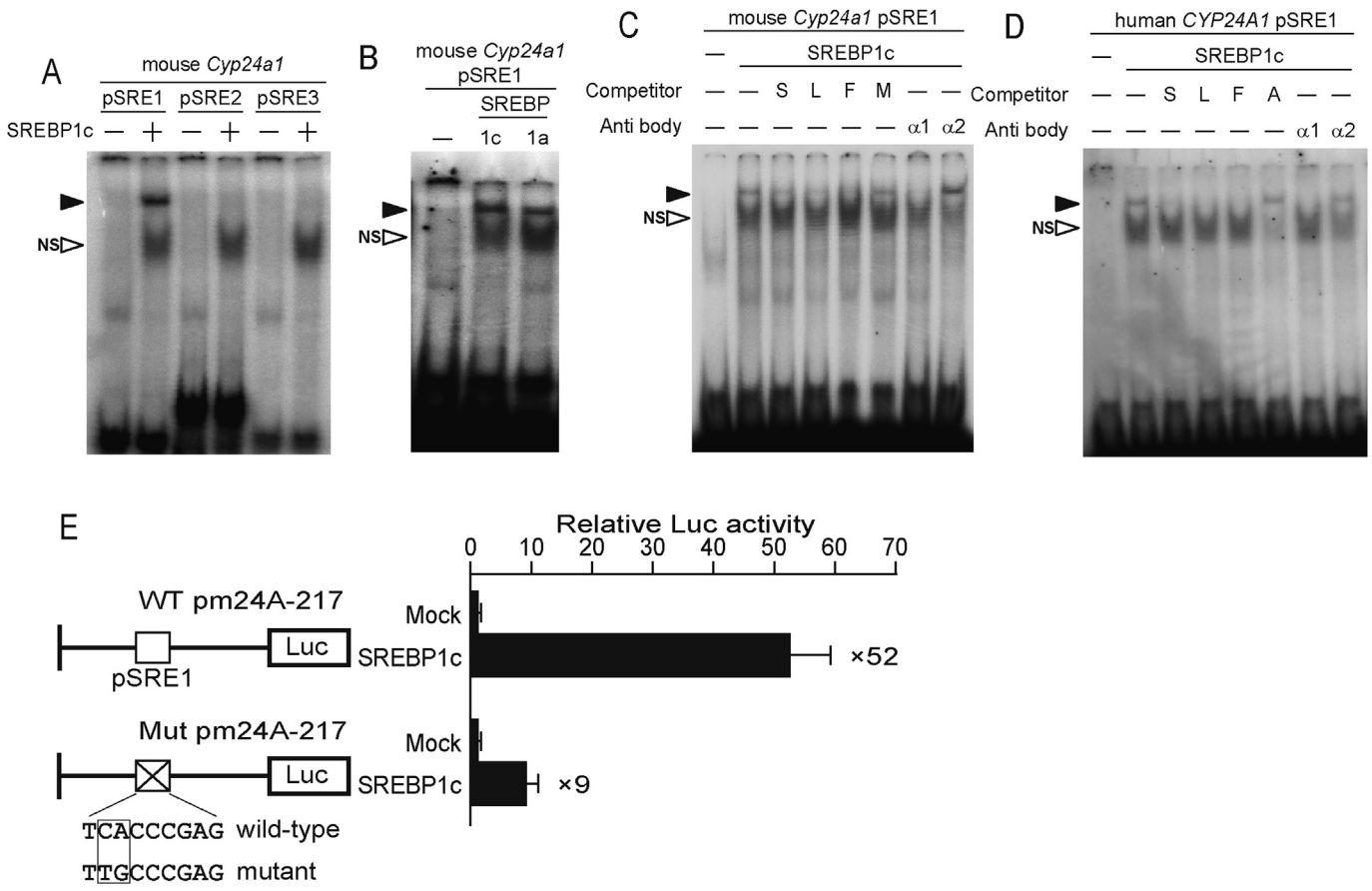


Fig. 3. pSRE1 is a cis-element for SREBP1 trans-activation. A) EMSAs performed using ^{32}P-labeled sequences containing pSRE1, pSRE2 or pSRE3 as probes with *in vitro*-synthesized human *SREBP1c* (amino acids 1–449). B) EMSAs performed using ^{32}P-labeled pSRE1 as probes with *in vitro*-synthesized human *SREBP1c* (amino acids 1–449) or human *SREBP1a* (amino acids 1–473). C, D) EMSAs performed using ^{32}P-labeled sequences containing mouse or human pSRE1 as probes with *in vitro*-synthesized human *SREBP1c* (amino acids 1–449). Unlabeled competitor oligonucleotides were added as indicated. A 100-fold molar excess of each competitor was used. S: Self; F: rat *Fasn* SRE; L: human *LDLR* SRE; M: mutated mouse *Cyp24a1* pSRE1; A: consensus AP1 responsive element; α1: SREBP1-specific antibody; α2: c-Fos-specific antibody. Arrows indicate the locations of the DNA-protein complex bands. Shifted bands were separated from free probe using a 6% non-denaturing polyacrylamide gel. Representative gels are shown. NS, nonspecific band. E) Schematic diagram showing wild-type mouse *Cyp24a1* (pm24A-217) and SRE1-muated mouse *Cyp24a1* (pm24A-217 mut; mutated region is marked by an X) reporter plasmid. OK-P cells were co-transfected with either wild type or mutated *Cyp24a1* reporter plasmid with pCMV-β, pcDNA3.1-*SREBP1c* or empty vector. Cell lysates were assayed for *β-gal* and luciferase activity. All data are represented as means ± SD (n = 2).Similar results were obtained from two independent experiments.

similarly to SREBP1c suggests that SREBP1c is likely involved in controlling levels of vitamin D metabolites in renal tissue (Fig. 4E). Furthermore, the down-regulation of *CYP24A1* mRNA levels in siRNA-transfected HKC-8 cells indicates that changes in *SREBP1* expression could affect renal *Cyp24a1* levels (Fig. 4B). Interestingly, a recent report showed that 25 (OH) vitamin D impairs SREBP activation independently of VDR [13], indicating that levels of vitamin D and its metabolic enzymes could regulate lipid metabolism. Further studies are needed to confirm the relation between SREBP1 and vitamin D metabolism.

Here we showed that SREBP1 regulates *CYP24A1* transcription. Since CYP24A1 metabolizes 25(OH) vitamin D and 1,25$(OH)_2$D to inactive forms, it is notable that renal SREBP1 could regulate *CYP24A1* expression in lipogenic conditions. For instance, several reports suggest that obese people have decreased amounts of circulating 25 (OH) vitamin D or 1,25 $(OH)_2$D [14–16]. Moreover, increased renal *Cyp24a1* expression was seen in mice fed a high-fat diet [17]. These findings, together with those of the present study, indicate that SREBP1 up-regulates CYP24A1 expression in several metabolic disorders. Actually, some reports indicated that SREBP1 is associated with progressive renal diseases. Indeed, activated renal SREBP1c induced tubule damage and glomerulosclerosis in both diabetes mice fed a high fat diet and genetically obese animals [18–20]. Although the kidney is a crucial organ in vitamin D metabolism, there is limited information concerning renal SREBP1 and vitamin D metabolism.

Although both SREBP1 isoforms strongly activated *CYP24A1* and *Fasn-Luc* transcriptional activity, SREBP1a had a more pronounced effect than did SREBP1c (Fig. 1A–C). Because SREBP1a has a longer N-terminal transactivation domain, its trans-activation capacity is assumed to be stronger than that for the 1c isoform [21]. In fact, a previous report that assessed transgenic mice overexpressing individual SREBP1 isoforms indicated that SREBP1a transgenic mice were more susceptible to fatty liver [22]. Because *SREBP1a* mRNA is dominantly expressed in actively growing tissues such as thymus, spleen and intestines [23], it is conceivable that SREBP1a act mainly in several extra-renal organs as a local vitamin D regulator.

Results of a luciferase reporter assay using several length promoters showed that specific promoter regions (−217 to −122) were crucial elements in trans-induction of SREBP1 (Fig. 2A). Moreover, we found three putative SREs in the *Cyp24a1* promoter region (Fig. 2B). Several reports indicated that SRE elements can coordinate the binding elements NF-Y and Sp1 [24,25], which are thought to be essential for the transactivation activity of SREBP1 through formation of a co-activator complex [26]. A previous report indicated that the rat *Cyp24a1* promoter includes NF-Y (CCAAT

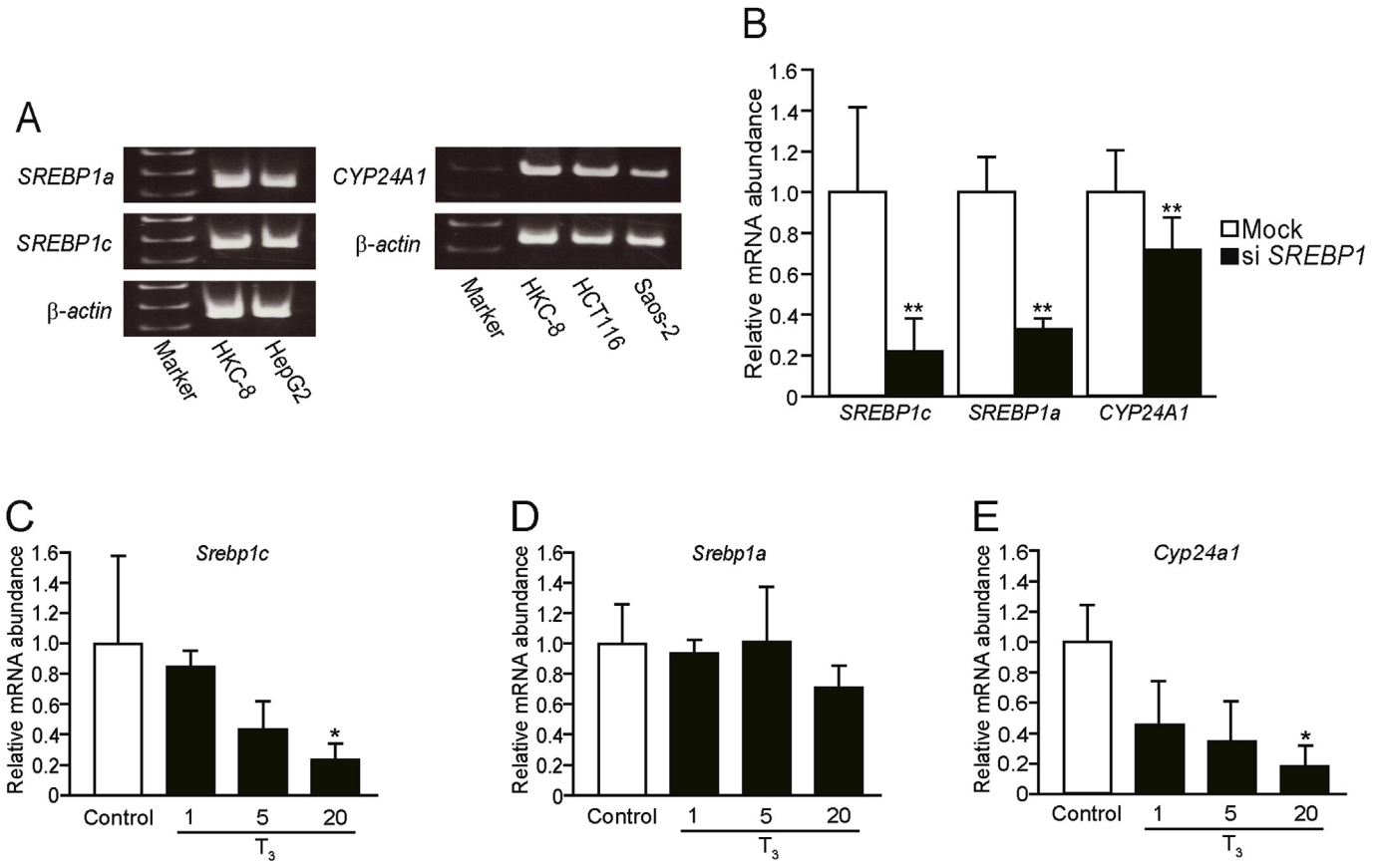


Fig. 4. Effect of *SREBP1* expression on *CYP24A1* mRNA levels in HKC-8 cells and mouse kidney. A) RT-PCR analysis of *SREBP1c*, *SREBP1a* and *CYP24A1* mRNA levels in indicated cell lines. B) HKC-8 cells were transfected with *SREBP1* specific siRNA or control siRNA, and incubated for 24 h. *SREBP1c*, *SREBP1a* and *CYP24A1* mRNA levels were determined by quantitative PCR. *β-ACTIN* was used as an internal control. All data are represented as means ± SD (n = 9–10). C-*E)* C57BL/6 mice were injected i.p. with T_3 (1, 5 or 20 μg/100 g body weight) or saline (control) and killed after 24 h. Renal C) *Srebp1c*, D) *Srebp1a*, and E) *Cyp24a1* mRNA levels were determined by quantitative PCR. *β-actin* was used as an internal control. All data are represented as means ± SD (n = 5). $^{*}p < 0.05$ vs. control, $^{**}p < 0.01$ vs. control.

box −62/-51) and Sp1(GC box −114/-101) binding elements in the proximal promoter region [27]. Here, 1,25$(OH)_2D_3$ additionally increased transcriptional activity of the *Cyp24a1* reporter plasmid pm24A-960 in the presence of SREBP1c (Fig. 1D).These findings suggest that multiple transcriptional factors including SREBP1 and VDR cooperate in *CYP24A1* promoter.

Site-directed mutagenesis of SRE1 sites was associated with marked decreases in SREBP1c trans-induction (Fig. 3E). Moreover, EMSA results indicated that mutated pSRE1 could not inhibit the binding of SREBP1c to labeled probes (Fig. 3C). These results support the direct binding of SREBP1 to pSRE1 and that this binding enhances *CYP24A1* promoter activity.

In conclusion, our results revealed that SREBP1 trans-activates *CYP24A1* expression that is likely mediated through a novel SREBP binding element within the mouse and human *CYP24A1* promoter.

Funding sources

This work was supported by the Ministry of Education [grants number 25282022 (to H.Y.)].

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Shoko Ikeda, Nozomi Yokoyama, Kotaro Abe, Rina Onishi, Kazuki Fujimoto and Shiori Fukuda (Department of Clinical Nutrition and Food Management, Institute of Biomedical Sciences, University of Tokushima Graduate School, Tokushima, Japan) for technical assistance.

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