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Identification and functional characterization of human glycerol-3-phosphate acyltransferase 1 gene promoters

Nagakatsu Harada *, Erika Fujimoto, Maiko Okuyama, Hiroshi Sakaue, Yutaka Nakaya

Department of Nutrition and Metabolism, Institute of Health Biosciences, University of Tokushima Graduate School, 3-18-15, Kuramoto-cho, Tokushima 770-8503, Japan

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ABSTRACT

Glycerol-3-phosphate acyltransferase 1 (GPAT1) acts as a rate limiting enzyme in triacylglycerol and phospholipid synthesis in mammals. GPAT1 regulates hepatic lipid accumulation associated with metabolic disorders. Here we have identified two transcriptional initiation sites and two promoters (promoter I and II) required for expression of the human GPAT1 (hGPAT1) gene. Promoter I regulates transcription of three alternative hGPAT1 mRNA variants, hGPAT1-V1, V2, and V3, while promoter II induces expression of a fourth variant, hGPAT1-V4. RT-PCR analysis and luciferase reporter assays revealed that promoter II acts in lipogenic tissues like the liver (and liver-derived HepG2 cells), whereas promoter I is differentially regulated and also acts in non-liver HeLa cells. Among liver-enriched transcription factors, HNF4α and C/EBPα slightly activated hGPAT1 promoter I, while factors including HNF1α altered promoter II activity. The lipogenic transcription factor SREBP1c greatly increased promoter II activity in HepG2 cells. The use of various truncated or mutated fragments of promoter II revealed that one sterol regulatory element-like motif and one inverted CCAAT box on promoter II contributed to the SREBP1c response. These cis-acting elements and trans-acting factors can be potential targets for manipulation of hepatic GPAT1 levels in humans.

1. Introduction

Glycerol-3-phosphate acyltransferase 1 (GPAT1) is one of the rate limiting enzymes in *de novo* glycerophospholipid (triacylglycerol and phospholipids) synthesis [1,2]. GPAT1 facilitates formation of 1-acylglycerol-3-phosphate (lysophosphatidic acid) by catalyzing the esterification of glycerol-3-phosphate with a fatty acyl-CoA [1,2]. The GPAT1 activity accounts for about 30–50% of total GPAT activity in the liver, while its activity is less than 10% of the total in other tissues, where the remaining GPAT isoforms (GPAT2, 3, and 4) play roles [1,2]. Therefore, relatively higher expression levels of GPAT in the liver induced by exogenous GPAT1 gene transfer [3] or obesity [4] result in high accumulation of triacylglycerol and hepatic steatosis in experimental animals.

The promoter sequences of GPAT1 have already been identified in mouse [5,6] and rat [7]. Both species have at least two promoters (promoters 1a and 1b in mouse, and the "distal" and "proximal" promoters in rat) for GPAT1 gene expression [6,7]. Mouse promoter 1a and the rat "distal" promoter are highly homologous [6,7]. These promoters have increased activities in response to binding of the sterol regulatory element-binding protein 1 (SREBP1), a lipogenic transcription factor which is activated upon food (carbohydrate) consumption [6,8]. Thus, hepatic expression levels of GPAT1 have been reported to be increased by feeding or insulin stimulation in these animals [6,9]. In contrast, depleting or reducing the consumption of food decreased hepatic GPAT1 expression in both lean and obese diabetic mice [6].

Transgenic mice overexpressing the liver-enriched transcription factor hepatocyte nuclear factor 3β (HNF3β) also showed elevated mRNA levels of GPAT1 gene in the liver [10]. This indicates that the mouse GPAT1 promoters might be responsive to HNF3s. However, whether the same regulations hold true for the human GPAT1 (hGPAT1) gene remains unclear. A recent study has demonstrated that adenovirus-mediated overexpression of HNF3α (also known as Foxa1) decreased hGPAT1 mRNA levels and lipid accumulation in human liver cells including HepG2 cells [11]. These observations imply that cis-acting elements and trans-acting factors on hGPAT1 promoter(s) play roles in the regulation of human lipid metabolism.

A DNA sequence with high homology to both the promoter 1a (in mouse) and the "distal" promoter (in rat) exists in the human genome [7]. However, neither the net promoter sequences nor the transcriptional initiation sites for the hGPAT1 gene have been determined experimentally. Identification of a common promoter among mammalian species (human, rat and mouse) in addition to a human-specific promoter(s) for the hGPAT1 gene is essential in applying the results of animal studies for GPAT1 inhibition to the treatment of human diseases with high lipid accumulation. In

* Corresponding author. Fax: +81 88 633 7113.
E-mail address: harada@nutr.med.tokushima-u.ac.jp (N. Harada).

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the present study, we have identified two transcriptional initiation sites and two expression promoters required for expression of the hGPAT1 gene. In addition, the effect of liver-enriched transcription factors on hGPAT1 promoter activity was examined using human cells *in vitro*.

2. Materials and methods

2.1. Cell culture

HepG2 cells, HeLa cells, and NIH/3T3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 5.5 mM glucose, 10% fetal bovine serum (FBS), and 1% penicillin and streptomycin.

2.2. Preparation of total RNA and genomic DNA

Total RNA was extracted from human cell lines with TRIzol reagent (Invitrogen, Carlsbad, CA). Human genomic DNA was isolated from HepG2 cells using the PUREGENE® Genome DNA Purification kit (Gentra Systems, Minneapolis, MN). Total human RNA from liver and kidney were purchased from CLONTECH (Palo Alto, CA). Total human RNA from adipose tissue was purchased from BioChain Institute, Inc (Hayward, CA).

2.3. 5′-Rapid amplification of cDNA ends (RACE)

Amplification of the 5′ end of hGPAT1 transcripts was performed using the GeneRacer™ Kit (Invitrogen) on human liver mRNA. A GeneRacer™ RNA oligonucleotide adaptor (5′-CGACUGGAGCACGAGGACACUGACAUGGACUGAAGGAGUAGAAA-3′) was ligated to the 5′ end of decapped mRNA using T4 RNA ligase (provided in the kit). First strand cDNA synthesis was then carried out using random primers (provided in the kit). hGPAT1-specific primers, 5′-ACGGCTTTTGATTGCTGCTGGGCAGA-3′ (nucleotide position +463 to +488 relative to the translation initiation site) and 5′-GGGTTTAA TTCAGCAGCCACTTCTGC-3′ (nucleotide position +430 to +455 relative to the translation initiation site) and adaptor-specific primers (provided in the kit) were used in the primary and secondary PCR rounds with the Ex-Taq DNA polymerase (TaKaRa, Kyoto, Japan). The resulting PCR products were cloned into the pCR®2.1-TOPO vector of the TOPO TA Cloning Kit (Invitrogen) and the nucleotide sequence of the cDNA fragments (5′ of hGPAT1-V1, V2, V3, and V4) was confirmed with an ABI PRISM 3100-*Avant* Genetic Analyzer (Applied Biosystems, Foster, CA) using a BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems), following the manufacturer's protocol.

2.4. Bioinformatics analysis

BLAST-N searching against the human genome (http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid = 9606) was performed with the DNA sequences obtained from the 5′-RACE analysis (described above) to analyze the chromosomal localization and exon/intron organization of the hGPAT1 gene. Genomatix software (http://www.genomatix.de/cgi-bin/matinspector_prof/mat_fam.pl?s = a3459775aee21e75b3cc7d2c65efd24d) was used for prediction of transcription factor binding sites on hGPAT1 promoters.

2.5. RT-PCR

Total RNA from human tissues and cultured cells was reverse transcribed using the PrimeScript® 1st strand cDNA Synthesis kit (TaKaRa). The subsequent PCR reaction was carried out using the Ex-Taq DNA polymerase (TaKaRa). For amplification of the hGPAT1-V1, V2, and V3 variants, forward, we used 5′-GCTCAGTG CGTGTCTTCAGC-3′ (primer S1, designed at exon 1a), and for reverse we used 5′-TCAGTTTCGGGTGCAGAATG-3′ (primer A1, designed at exon 1e). For amplification of the hGPAT1-V4 variant, forward, we used 5′-CCAGAGGAACCAGACCTTGC-3′ (primer S2, designed at exon 1d), and for reverse we used the primer A1. The sizes of the PCR products for hGPAT1-V1, V2, V3, and V4 variants were 417, 319, 209, and 171 base pairs, respectively. For amplification of the β-actin gene (228 base pairs), forward, we used 5′-CCAGAGCAAGAGAGGCATCC-3′, and for reverse we used 5′-ACGTACA TGGCTGGGGTGTT-3′. The number of cycles for the PCR reaction was adjusted to 35 and 28 for hGPAT1 and β-actin, respectively. The PCR products were then electrophoresed on a 6% polyacrylamide gel.

2.6. Construction of luciferase reporter plasmids

The 5′-flanking DNA fragments encoding nucleotides −1979 to +136 of the hGPAT1 exon 1a and −2022 to +107 of the hGPAT1 exon 1d (relative to the transcription initiation site) were generated by PCR using human genomic DNA and the specific primers listed in Supplementary Table S1. PCR products were subcloned with the TOPO TA Cloning Kit (Invitrogen) and the nucleotide sequence of each DNA fragment was confirmed as described above. Each DNA insert was isolated with restriction enzymes (Supplementary Table S1) and inserted into the same restriction sites of the pGL3-basic luciferase reporter vector (Promega, Madison, WI), generating PGL I (−1979) and PGL II (−2022) plasmids. PGL II (−1329) was constructed by digesting PGL II (−2022) plasmid with BlnI/HindIII, and inserting the fragment into the NheI/HindIII site of the pGL3-basic vector. PGL II (−654) was generated by cutting out a 1.38-kb NheI fragment from PGL II (−2022), followed by self-ligating the remaining fragment at the NheI site. PGL II (−235) was constructed by cutting out three (1.24-kb, 0.36-kb, and 0.22-kb) SacI fragments from the PGL II (−2022) plasmid, followed by self-ligating the remaining fragment at the SacI site. The 5′-flanking DNA fragments encoding nucleotides −91 to +107, or −60 to +107 of the hGPAT1 exon 1d (relative to the transcription initiation site), were generated by PCR using PGL II (−235) plasmid as a template. The specific primers are listed in Supplementary Table S1. PCR products were subcloned and inserted into the pGL3-basic vector (Promega), generating PGL II (−91) and PGL II (−60) plasmids. PGL II (−91)m1, PGL II (−91)m2, and PGL II (−91)m3 were constructed by the same method as PGL II (−91), but using the specific mutant (sense) primers listed in Supplementary Table S1.

A luciferase reporter plasmid encoding nucleotides −1797 to +103 of the mouse GPAT1 (mGPAT1) exon 1a [termed PGL 1a (−1797)] and −2107 to +152 of the mGPAT1 exon 1b [(termed PGL 1b (−2107)] (relative to the transcription initiation site) was reported previously [6]. All plasmids were purified with a plasmid purification kit (QIAGEN, Valencia, CA) before use.

2.7. Construction of mammalian expression plasmids encoding a transcription factor gene

A cDNA fragment encoding the full open reading frame of human HNF1α (GenBank accession number NM_000545), human HNF1β (NM_000458), human HNF3α (NM_004496), human HNF3β (NM_153675), or human HNF4α (NM_000457) was amplified by RT-PCR using RNA isolated from HepG2 cells and the gene-specific primers listed in Supplementary Table S2. All PCR products included an intrinsic translational termination codon. The PCR product was subcloned into a PCR®2.1-TOPO vector (Invitrogen). A sequence-confirmed cDNA fragment was isolated with restriction enzymes (Supplementary Table S2) and inserted into the same restriction

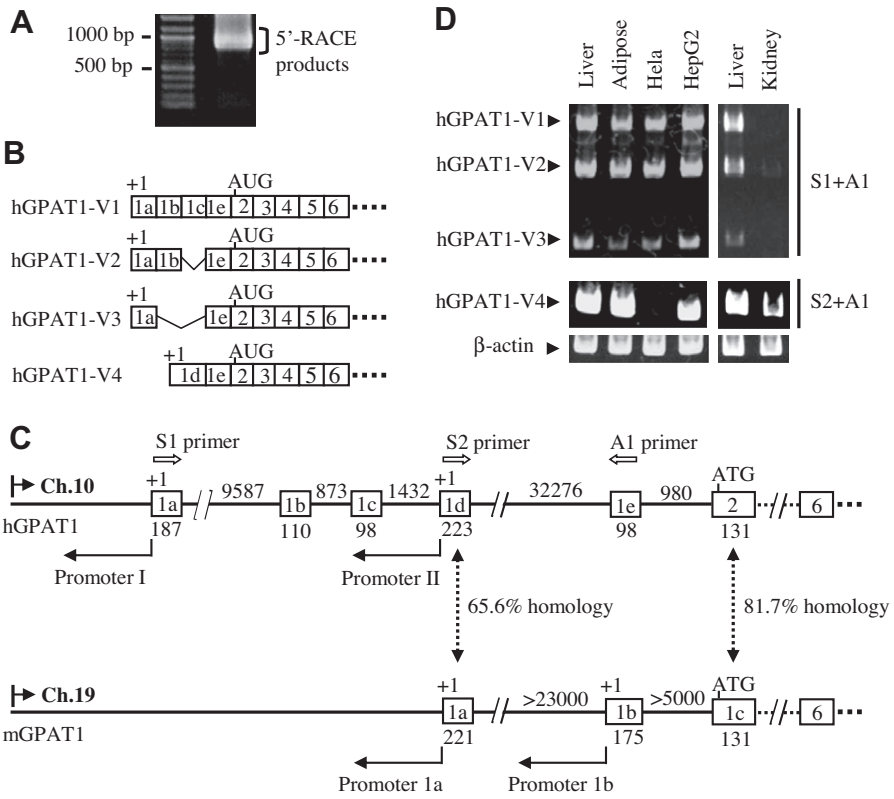

Fig. 1. Transcript variants and genomic organization of the human GPAT1 (hGPAT1) gene. (A) Agarose gel electrophoresis of 5′-RACE products of the hGPAT1gene derived from human liver. (B) Structures of the hGPAT1 transcript variants (hGPAT1-V1, V2, V3, and V4) obtained by 5′-RACE analysis. Boxes indicate the exons. The transcription initiation sites (+1) and the translational initiation codon (AUG) are indicated. (C) Promoter regions and exon/intron organization of the hGPAT1 (top) and mouse GPAT1 (mGPAT1) (bottom) gene [6]. The hGPAT1 is mapped to Chromosome (Ch.) 10, whereas mGPAT1 is located in Ch. 19. Boxes indicate the exons. The numbers of nucleotides in each exon and intron are shown. The transcription initiation sites (+1) and the translational initiation codon (ATG) are indicated. The locations of promoter I and II for expression of hGPAT1 transcript variants (see panel B) and promoter 1a and 1b for expression of mGPAT1 transcript variants [6] are shown. Open arrows indicate the locations of the primers used for RT-PCR analysis (panel D). Nucleotide sequence homology (%) between hGPAT1 and mGPAT1 exons is shown by a dotted arrow. (D) Expression of hGPAT1 transcript variants in human tissues and cultured cells (RT-PCR). β-actin was used as an internal control.

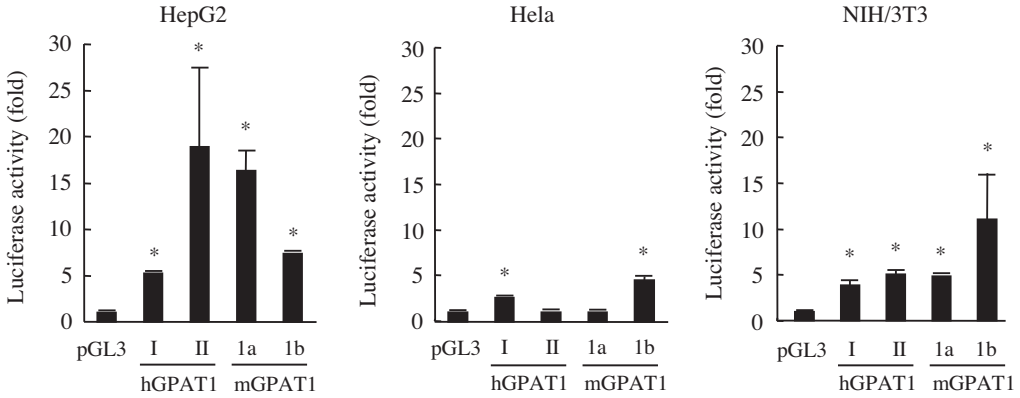

Fig. 2. Transcriptional activities of hGPAT1 promoters (I and II) and mGPAT1 promoters (1a and 1b) in cultured cells. HepG2, HeLa, or NIH/3T3 cells were transfected with one of the luciferase reporter plasmids [for hGPAT1 promoters: PGL I (−1979) (I) or PGL II (−2022) (II); for mGPAT1 promoters: PGL 1a (−1797) (1a) or PGL 1b (−2107) (1b)] and luciferase activities were evaluated 48 h post-transfection. Luciferase activity was normalized for β-gal activity. A pGL3-basic vector (pGL3) was used as a promoter-less control. Data are expressed as the means ± SD of four culture wells (in a 24-well plate) in each group. The mean value for the promoter-less control (pGL3) is designated as 1. *$P < 0.05$ vs. pGL3.

sites of the pcDNA3.1/Myc-His (+) vector (Invitrogen) without fusion to the epitope tags (*myc* and 6xHis tags) encoded in the vector.

A cDNA fragment encoding an active fragment of human SREBP1c (amino acids 1–466) was also amplified by RT-PCR using specific primers listed in Supplementary Table S2. PCR product was

subcloned as described above and inserted into the pcDNA3.1/Myc-His (+) vector (Invitrogen) using EcoRI.

The pcDNA3.1 plasmids, including mouse CCAAT/enhancer binding proteinα (C/EBPα) or C/EBPβ cDNA, were reported previously [12].

2.8. Transfection and luciferase assay

Cells grown in 24-well plates were transiently transfected with 0.20 μg of the luciferase reporter plasmid and 0.10 μg of the pcDNA3.1 plasmid (with or without the transcription factor cDNA) using the Lipofectamine™ 2000 reagent (Invitrogen). The normalized transfection efficiency for luciferase activity was determined by co-transfecting with 0.10 μg of β-galactosidase (β-gal) expression vector, pCMV-β (Stratagene, La Jolla, CA). After 48 h, cells were harvested in a lysis buffer supplied with the luciferase assay kit (Promega) and the lysates were assayed for luciferase activity and β-gal activity as described previously [13].

2.9. Statistical analysis

Data are expressed as the means ± SD. Data were analyzed by Student's *t* test and one-way analysis of variance. A *P*-value < 0.05 was accepted as statistically significant.

3. Results and discussion

3.1. Transcript variants and exon/intron organization of the hGPAT1 gene

To determine the transcription initiation sites of the hGPAT1 gene, 5′-RACE analysis was carried out using hGPAT1-specific reverse primers as described in Materials and methods. Sequencing of the 5′-RACE products (Fig. 1A) revealed four transcript variants which were designated hGPAT1 transcript variant 1 (hGPAT1-V1), variant 2 (hGPAT1-V2), variant 3 (hGPAT1-V3), and variant 4 (hGPAT1-V4).

The exon/intron organization of the hGPAT1 gene was analyzed by NCBI genome search programs using the obtained 5′-sequences of the four hGPAT1 transcript variants as probes. As shown in Fig. 1C, the hGPAT1 gene possesses five 5′-untranslated exons (exons 1a to 1e). The translational initiation codon (ATG) is located at exon 2. All sequences at the exon–intron junctions are consistent with the AG-GT rule (not shown). The transcription initiation site (+1) of hGPAT1-V1, V2, and V3 is located at exon 1a (Fig. 1C). hGPAT1-V1, V2, and V3 cDNA contained four (1a, 1b, 1c, and 1e), three (1a, 1b, and 1e), and two (1a and 1e) untranslated exons, respectively, in their 5′-terminal regions (Fig. 1B). The transcription initiation site (+1) of the hGPAT1-V4 variant was located at exon 1d, which is approximately 12 kb downstream of exon 1a (Fig. 1C). The hGPAT1-V4 cDNA contained two (1d and 1e) untranslated exons in its 5′-terminal region (Fig. 1B). The DNA region upstream of exon 1a was designated as promoter I (nucleotide sequence is shown in Supplementary Fig. S1) and that upstream of exon 1d as promoter II (nucleotide sequence is shown in Supplementary Fig. S2) for hGPAT1 gene transcription (Fig. 1C).

3.2. Expression of hGPAT1 mRNA variants in human tissues and cultured cells

The expression of each hGPAT1 transcript variant (hGPAT1-V1, V2, V3, and V4) from various human tissues and cultured cells was examined by RT-PCR. As shown in Fig. 1D, the promoter I-dependent transcripts (hGPAT1-V1, V2, and V3) were expressed in human liver, adipose tissues, HepG2, and HeLa cells, but not in the kidney. The expression of the promoter II-dependent hGPAT1-V4 transcript was observed in human liver, adipose tissues, kidney, and HepG2 cells, but not in HeLa cells.

3.3. Comparison of GPAT1 gene organization between human and mouse

Fig. 1C shows the genomic organizations of the GPAT1 gene in human (hGPAT1) and mouse (mGPAT1). Homology analysis for the exon sequences revealed relatively high homology of 65.6% between exon 1d in hGPAT1 and exon 1a in mGPAT1, and of 81.7% between exon 2 in hGPAT1 and exon 1c in mGPAT1. The DNA sequence of promoter II in hGPAT1 was highly homologous to mGPAT1 promoter 1a (Supplementary Fig. S2) or rat GPAT1 "distal" promoter (data not shown). These results suggest that the promoter region corresponding to promoter II (human), promoter 1a (mouse), and the "distal promoter" (rat) is highly conserved across mammals, and that they might play significant roles in GPAT1 gene transcription in lipogenic tissues. We could not find a promoter I-homologous region in mouse (Fig. 1D) or in rat DNA (not shown).

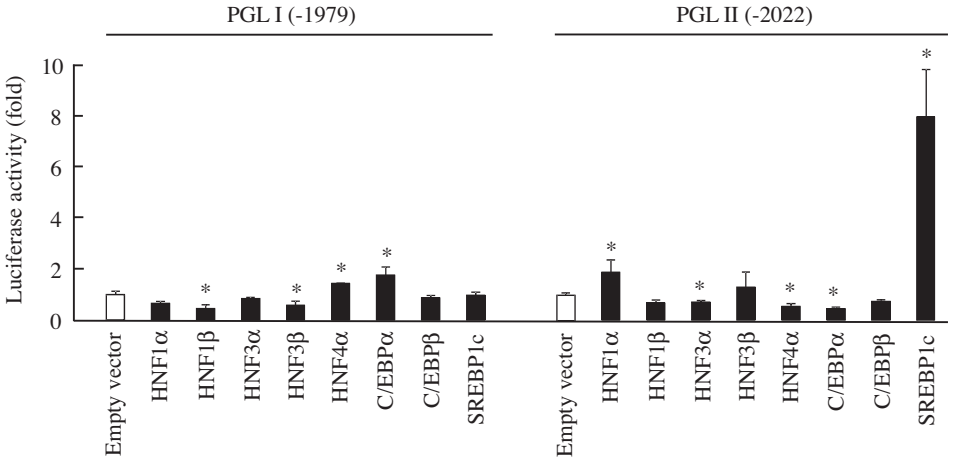

Fig. 3. Effect of liver-enriched transcription factors on hGPAT1 promoter activity. HepG2 cells were transfected with one of the luciferase reporter plasmids [PGL I (−1979) for hGPAT1 promoter I or PGL II (−2022) for hGPAT1 promoter II] together with a transcription factor (as indicated)-encoding or a non-coding (empty vector) plasmid. Luciferase activities were evaluated 48 h post-transfection. Luciferase activity was normalized for β-gal activity. Data are expressed as the means ± SD of four culture wells (in a 24-well plate) in each group. The mean value for the empty vector group is designated as 1. *$P < 0.05$ vs. empty vector group.

Thus, promoter I might play a role in human-specific regulation of the GPAT1 gene.

3.4. Promoter activities of the human and mouse GPAT1 gene in cultured cells

We constructed a series of GPAT1 promoter-driven luciferase reporter plasmids for this study. First we evaluated the activities of hGPAT1 promoter I [−1979 to +136 in PGL I (−1979)] and promoter II [−2022 to +107 in PGL II (−2022)], as well as mGPAT1 promoter 1a [−1797 to +103 in PGL 1a (−1797)] and promoter 1b [−2107 to +152 in PGL 1b (−2107)] (relative to the transcription initiation site) in three different cell-lines by luciferase assay. As shown in Fig. 2, luciferase activities derived from the hGPAT1 promoter II- or mGPAT1 promoter 1a-including reporter plasmid were much higher (16–19 folds) than the activities derived from the promoter-less empty plasmid (control) group in liver-derived HepG2 cells. However, such a large increase in luciferase activity was not observed in non-liver HeLa cells or NIH/3T3 cells. Luciferase activities derived from the hGPAT1 promoter I-including reporter plasmid were 5.2-fold (HepG2 cells), 2.7-fold (HeLa cells), and 3.8-fold (NIH/3T3 cells) that of the control cells, whereas those derived from the mGPAT1 promoter 1b-including reporter plasmid were 7.3-fold (HepG2 cells), 4.5-fold (HeLa cells), and 11.0-fold (NIH/3T3 cells) those of the control groups (Fig. 2).

3.5. Effect of liver-enriched transcription factors on hGPAT1 promoter activity

To clarify the molecular mechanisms regulating the activity of promoter II in HepG2 cells (Fig. 2), we investigated the effect of liver-enriched transcription factors including HNF1 (HNF1α and HNF1β), HNF3 (HNF3α and HNF3β), HNF4α, C/EBP (C/EBPα and C/EBPβ) and SREBP1c [14–18] on hGPAT1 promoter activity. As shown in Fig. 3, overexpression of HNF4α or C/EBPα slightly but significantly increased promoter I activity (1.44 or 1.76-fold, respectively). Overexpression of HNF1α increased promoter II activity by 1.88-fold (Fig. 3). It has been reported that HNF1α and HNF3β cooperatively activate the promoter of glucose transporter type 2, a gene predominantly expressed in hepatocytes and pancreatic β-cells [19]. HNF3β is also known to increase hepatic mGPAT1 mRNA levels in mouse [10]. However, HNF3β overexpression did not increase

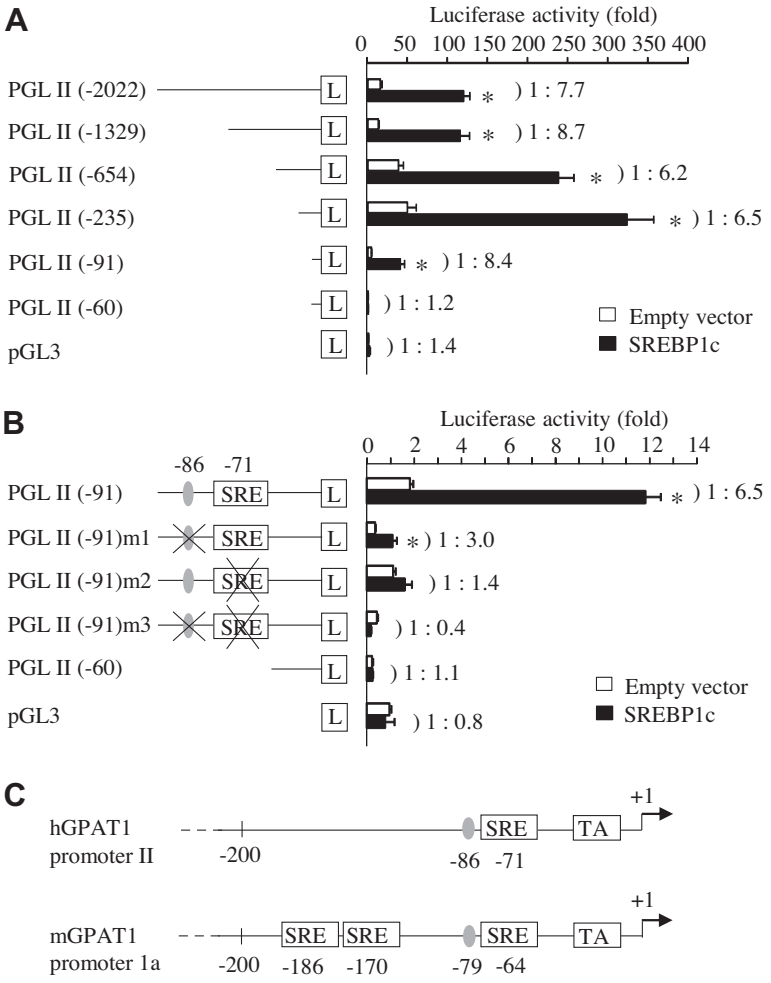

Fig. 4. Regulation of hGPAT1 promoter II activity by SREBP1c. (A and B) HepG2 cells were transfected with one of the luciferase reporter plasmids (as indicated) together with a SREBP1c-encoding or a non-coding (empty vector) plasmid. Luciferase activities were evaluated 48 h post-transfection. A pGL3-basic vector (pGL3) was used as a promoter-less control. The locations of the SRE-like motif (SRE) at −71 and the inverted CCAAT box (gray circle) at −86 (relative to the transcription initiation site) on promoter II are shown in panel B. Site-directed mutagenesis resulted in the disruption of each motif (indicated by an X). Luciferase activity was normalized for β-gal activity. Data are expressed as the means ± SD of four culture wells (in a 24-well plate) in each group. The mean value for a promoter-less control (pGL3) with an empty vector is designated as 1. $^{*}P < 0.05$ vs. empty vector group. The ratios of the luciferase activities between empty vector and SREBP1c groups are indicated. L, luciferase. (C) Locations of SREBP1c-responsive sequences in hGPAT1 promoter II (top) and mGPAT1 promoter 1a (bottom). The transcription initiation site is indicated as +1. The SRE-like motif or the inverted CCAAT box is indicated as SRE and a gray circle, respectively. The numbers relative to the transcription initiation site are shown. TA, TATA box.

hGPAT1 promoter II activity regardless of the presence of HNF1α in this study. Other transcription factors, including HNF3α, did not increase hGPAT1 promoter activity (Fig. 3). HNF3α actually decreased promoter II activity (Fig. 3), which might explain the decrease of hGPAT1 mRNA levels in HNF3α-overexpressing human hepatocytes [11]. Predicted binding sites for HNFs or C/EBPs on each hGPAT1 promoter are shown in Supplementary Fig. S1 (promoter I) and Supplementary Fig. S2 (promoter II). Overexpression of SREBP1c yielded the highest activation of hGPAT1 promoter II (8.0-fold), suggesting a central role for SREBP1c in regulating GPAT1 expression in human liver cells (Fig. 3).

3.6. Identification of the SREBP1c-responsive sequence in hGPAT1 promoter II

Various 5′-segments for promoter II (from −2022, −1329, −654, −235, −91, and −60 to +107) flanking the transcriptional initiation site were inserted into a luciferase reporter plasmid and responses of these DNA regions to SREBP1c overexpression were evaluated in HepG2 cells. As shown in Fig. 4A, SREBP1c induced a large increase (ranging from 6.2 to 8.7-fold) in luciferase activity when cells were transfected with a PGL II (−2022), (−1329), (−654), (−235), or (−91) plasmid. The luciferase activity of HepG2 cells transfected with the pGL II (−60) plasmid, however, was not significantly affected by SREBP1c overexpression (Fig. 4A). We hypothesized that SREBP1-responsive sequences are located between −91 and −60 (relative to the transcription initiation site) in promoter II.

Previous studies showed that the mGPAT1 promoter 1a contains three sterol regulatory element (SRE)-like sequences (SRE1: −186, SRE2: −170, and SRE3: −64) and one inverted CCAAT box (−79) (relative to the transcription initiation site) within 200 bases upstream of the transcriptional initiation site [8]. The SRE-like sequences have been shown to bind SREBP1 protein [8]. The inverted CCAAT box binds an NF-Y transcription factor and plays a critical role in SREBP1-induced activation of mGPAT1 transcription [8]. One SRE-like sequence at −71 and one inverted CCAAT box at −86 as well as the physical distance (10 base pairs) between them are perfectly conserved in promoter II of the hGPAT1 gene (Fig. 4B and C, and Supplementary Fig. S2). However, no additional SRE (-like) sequences were found in the promoter II region within 200 nucleotides upstream of the transcription initiation site (Fig. 4C). As shown in Fig. 4B, disruption of the SRE-like sequence (−71), inverted CCAAT box (−86), or both decreased or abolished the SREBP1c-stimulated increase in promoter II activity, suggesting that these motifs act as SREBP1c-responsive sequences.

3.7. SREBP1c can be a potential target for manipulation of hepatic GPAT1 levels in humans

It has been reported that obese mice have elevated levels of SREBP1c and mGPAT1 mRNA in the liver [4,20], although another study failed to show this phenomenon [6,21]. On the other hand, disruption of SREBP1c in genetically obese *ob/ob* mice resulted in the reduction of hepatic mGPAT1 levels and ameliorated hepatic steatosis, although the obese condition and systemic insulin resistance in these mice were not improved [22]. Gene knockout of mGPAT1 itself showed similar effects in *ob/ob* mice [23]. Our observation that the expression of hGPAT1 also depends on SREBP1c suggests that SREBP1c as well as GPAT1 itself might be a useful target for the treatment of GPAT1-dependent hepatic steatosis in humans.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.05.094.

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