Interrelationship between LXRα, SREBP-1c, PPARγ and SHP in the transcriptional regulation of glucokinase gene expression in liver

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Directed by Professor Yong-Ho Ahn

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ABSTRACT

Interrelationship between LXRα, SREBP-1c, PPARγ and SHP in the transcriptional regulation of glucokinase gene expression in liver

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(Directed by Professor **Yong-Ho Ahn**)

Hepatic glucokinase (LGK) plays an essential role in controlling blood glucose levels and maintaining cellular metabolic functions in liver. Expression of LGK is mainly regulated by insulin through sterol regulatory element binding protein-1c (SREBP-1c) as a mediator. Since glucokinase expression is known to be decreased in the liver of liver X receptor (LXR) knock out mice, we have investigated whether glucokinase might be directly

activated by LXRα. Furthermore, we have studied interrelationship between transcription factors that control gene expression of LGK. In the current studies, we demonstrated that LXRα increased LGK mRNA level in primary hepatocytes and that there is a functional LXRE in the LGK gene promoter as shown by electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) assay. In addition, our studies demonstrate that LXRα and insulin activation of the LGK gene promoter occurs through a multi-faceted indirect mechanism. It is reported that LXRα increases SREBP-1c mRNA expression and then insulin stimulates the processing of the membrane bound precursor SREBP-1c protein and it moves to the nucleus and activates LGK expression through SREBP sites in its promoter. LXRα also activates the LGK promoter by increasing the transcriptional activity of peroxisome proliferators-activated receptor (PPAR)-γ, which also stimulates LGK expression through a PPRE. This activation is tempered through a negative mechanism where small heterodimer partner (SHP) decreases LGK gene expression by inhibiting the transcriptional activity of LXRα and PPARγ by directly interacting with their common heterodimer partner RXRα. From these data, we propose a mechanism for LXRα in controlling the gene expression of LGK that involves activation through SREBP-1c and PPARγ

and inhibition through SHP.

Key words: LXRα, SREBP-1c, PPARγ, SHP, Glucokinase,

Glucose metabolism

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I. INTRODUCTION

Several tissues are involved in maintaining optimal blood glucose levels. Among these, liver plays a major role by maintaining the balance between the storage and release of glucose[1]. Conversion of glucose to glucose-6-phosphate is a rate controlling reaction in the hepatic glucose metabolism. If the hepatic glycogen stores are replete, glucose-6-phosphate enters the glycolytic

pathway to produce pyruvate for *de novo* lipogenesis.

Glucose entering the liver is phosphorylated by hepatic glucokinase (LGK) (ATP: D-glucose 6-phosphotransferase, EC2.7.1.1). Since the K_m value of LGK is considerably higher than normal blood glucose concentrations and LGK is not subjected to allosteric regulation by the end product, the rate of glucose phosphorylation is directly proportional to the blood glucose level. Thus, LGK is considered to play an essential role for sensing and maintaining proper blood glucose levels [1, 2]. GK is mainly expressed in the liver, pancreatic β cells and neuroendocrine cells of the brain. Two alternate promoters regulate the tissue specific expression of the GK gene in the liver and pancreatic β cells [3]. An upstream promoter regulates β cell-specific GK (βGK) expression, whereas the promoter that regulates hepatic GK (LGK) gene expression is controlled by a downstream promoter. In the liver, LGK gene expression is regulated in response to fasting and refeeding [4], with insulin and glucagon serving as the mediators of this response. Insulin stimulates LGK gene expression in primary cultured hepatocytes regardless of glucose concentration, and glucagon inhibits LGK gene expression [5, 6]. The action of insulin on the upregulation of LGK transcription is mediated by the SREBP-1c [7, 8].

LXRs are nuclear receptors which sense oxysterols and regulate cholesterol and lipid metabolism [9, 10]. The LXR family consists of LXRα and LXRβ isoform. LXRα is expressed primarily in liver, adipose tissue, kidney, macrophage and intestine, whereas LXRβ is present ubiquitously [11]. LXRs form heterodimers with RXRα and bind to target DNA sequences known as the LXR response element (LXRE) [12]. In response to oxysterols, LXRs activate genes involved in reverse cholesterol transport, and hepatic cholesterol metabolism [13]. LXRα also increases the synthesis of fatty acids by either upregulating SREBP-1c or binding to the promoters of some lipogenic genes directly [14-16]. Other diverse roles of LXRα include the inhibition of the expression of gluconeogenic genes such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [17]. In addition, LXRα stimulates adipocyte differentiation through induction of PPARγ expression [18].

SHP is an atypical nuclear receptor that lacks a conventional DNA binding domain [19, 20]. It is known to interact with other transcriptional factors and represses transcriptional activity by either competing with other coactivators for binding to an affected transcription factor or by recruiting corepressors directly to the transcriptional repression domain of SHP [21]. SHP is expressed

in liver and plays an important role in the regulation of cholesterol homeostasis [22, 23]. SHP deficiency leads to improvement in diabetes by increasing insulin sensitivity through inducing of LGK gene expression [24]. Also, it is known that SHP regulates LXR transcriptional activity and augments the transcriptional activity of PPARγ [25, 26].

In this study, we identified an interactive mechanism for regulation of LGK expression through LXRα with critical roles for SREBP-1c, PPARγ and SHP. LXRα directly activated LGK expression by binding to the LXRE in LGK promoter and LXRα-mediated LGK gene expression was also indirectly upregulated by increasing SREBP-1c gene expression and increasing transcriptional activity of PPARγ. We also show that SHP inhibited LXRα dependent transcriptional activation of LGK by interacting with RXRα. These results suggest a multi-component mechanism for regulation of LGK expression by diverse nuclear receptors.

II. MATERIALS AND METHODS

Plasmids and materials

Construction of the luciferase reporter of the rat LGK promoter, pRGKL-1448, and its mutants m2, and mab were described earlier [8, 27]. Mutant clones, m4, m5, m6, and m7 were constructed by introducing substitution mutations into pRGKL-1448. Expression plasmids for PPARγ, RXRα, β-gal and their control vectors were previously described [8, 28]. Human SHP expression vector was a kind gift from Dr. Choi HS and LXRα expression vector was a kind gift from Dr. Mangelsdorf [29, 30]. The expression plasmid for *myc*-His-tagged pcLXRα was received from Dr. Kim JB [18]. LGK-PPRE×3tkLUC and LGK-LXRE×3tkLUC were produced by inserting three copies of the -119/-98 and -56/-32 regions of the LGK promoter into thymidine kinase (TK) minimal promoter in luciferase reporter (ptkLuc). Clones used in the mammalian two hybrid assay, pGal4-PPARγ, RXRα, SHP and pVP16-PPARγ, RXRα, SHP were subcloned by inserting the PCR amplified fragments containing PPARγ, RXRα and SHP into pM and pVP16 vectors (Clontech, Mountain View, CA). All of plasmid constructs were confirmed by DNA sequencing. Rosiglitazone and LG268 were each kind gift from GlaxoSmithKline Korea (Seoul, Korea)

and Timothy F. Osborne (UC, Irvine) and *9-cis* retinoic acid (9-CR), GW3965 and insulin were purchased from Sigma-Aldrich (St. Louis, MO). TO-901317 (T1317) was purchased from Cayman (Ann Arbor, Michigan).

Cell culture and transient transfection assay

Alexander cells (Human hepatoma cell lines, ATCC No. CRL8024) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin. The culture medium for Alexander cells was supplemented with 50 μg/mL tylosine for anti-PPLO agent. Primary cultured hepatocytes were isolated from Sprague-Dawley rats (~200 g) and cultured as described previously [8]. Transient transfection and luciferase assays were performed using Lipofectamine PLUS reagent (Invitrogen, Carlsbad, CA) and a luciferase assay kit (Promega, Madison, WI) [27]. Luciferase activities were normalized with β-galactosidase activities to adjust transfection efficiency. Normalized luciferase activities are shown as the mean ± S.D. of three independent experiments performed in triplicate and are expressed as fold increases relative to the basal activity of the reporters in the absence of overexpression vectors.

Preparation of recombinant LXRα protein and anti-LXRα antibody.

To prepare bacterial recombinant fusion proteins, pET-LXRα was transformed into *E.coli* (BL21-DE3). The recombinant LXRα was induced for 4 hr with 0.5 mM isopropyl-β-D-thiogalactopyranoside. The recombinant protein containing polyhistidine (His) tag was purified by Ni-NTA agarose resin chromatography (Peptron, Taejeon, Korea). One milligram of recombinant LXRα protein was suspended in 1 ml PBS and emulsified with 1 mM Freund's complete adjuvant (Sigma, St. Louis, MO) which was injected subcutaneously into the dorsal side of New Zealand White (NZW) rabbit.

Isolation of total RNA, reverse transcription, and quantitative polymerase chain reaction (qPCR).

Total RNA was extracted from primary cultured hepatocytes using Illustra RNAspin Mini RNA isolation kit (GE Healthcare, Buckinghamshire, UK) according to the manufacturer's protocol. First strand cDNA was synthesized from 2 μg of total RNA in a 20 μL volume using random hexamers and ImProm II Reverse transcriptase (Promega, Madison, WI). Real time qPCR was performed using the ABI PRISM 7000 Sequence Detection System instrumentation and software (Applied Biosystems, Foster City, CA)

according to the manufacturer's protocol with minor modifications. Briefly, the appropriate amount of the reverse transcription reaction mixture was amplified with specific primers using SYBR green PCR master mix (Applied Biosystems, Foster City, CA). Expression levels of target genes were determined by generating a five-point serial standard curve. This curve was used to calculate the amount of target gene mRNA in vehicle and ligand treated samples based on real time PCR. RNA samples were normalized by determining β-actin mRNA level. Primers used in PCR were as follow**:**

LGK-f, 5'-CTCAggAgTCAggAACATCT-3'

LGK-r, 5'-TgACCAgCATCACTCTgAAg-3'

SREBP-1c-f, 5'-ggAgCCATggATTgCACATT-3'

SREBP-1c-r, 5'-AggAAggCTTCCAgAgAggA-3'

FAS-f, 5'-AgCCTAACACCTCTgTgCAgT-3'

FAS-r 5'-TCCTTgCAgCCATCTgTgTTC-3'

LXRα-f, 5'-gAgAAgCTggTggCTgCCCA-3'

LXRα-r, 5'-AgCTgTAggAAgCCAgggAg-3'

PPARγ-f, 5'-TCCgTgATggAAgACCACTC-3'

PPARγ-r, 5'-CCCTTgCATCCTTCACAAgC-3'

β-actin-f, 5'-TTgTAACCAACTgggACgATATgg-3'

β-actin-r, 5'-CgACCAgAggCATACAgggACAAC-3'

Electrophoretic mobility shift assay (EMSA)

In vitro translated proteins of LXRα-myc/His and RXRα-flag were prepared by using a coupled transcription/translation kit (Promega, Madison, WI). Synthesis of full length LXRα-myc/His and RXRα-flag proteins were confirmed by western blotting. An oligonucleotide covering the -59/-29 region of the rat LGK promoter was used as a wild-type probe. The probes were labeled as previously described [31]. Ten pmol of single-stranded sense oligonucleotide was labeled with [γ-^{32}P] ATP using T4 polynucleotide kinase (New England Biolabs Inc., Ipswich, MA) and annealed with a 5 molar excess of antisense oligonucleotide. ^{32}P-labeled double-stranded oligonucleotides were purified with Sephadex G25 spin columns (Pharmacia, Piscataway, NJ). Ten thousand cpm (approximately 0.08 pmol) of probe was incubated with the *in vitro* translated protein for 30 min in 10 mM Hepes (pH 7.9, KOH), 1 mM $MgCl_2$, 30 mM KCl, 1 mM DTT, 5% glycerol. One μg of poly (*dI-dC*) was added to each reaction to suppress nonspecific binding. For competition assays, excessive unlabeled GLUT4 LXRE oligonucleotides [32] were added to the reaction mixture. Two ul anti-myc antibody were added to reaction for

supershift assay (Cell signaling, Denver, MA). The oligonucleotides used in EMSA were as follow:

wild-type, 5'-CTggCCCTgACCTTGTGACACTAggCAggg-3';

GLUT4 LXRE, 5'-CAgCCCCgggTTACTTTggggCATTgCTCC-3'

Chromatin immunoprecipitation (ChIP) assay

The ChIP assay was performed based on a previously described method with minor modification [33]. Proteins were cross-linked to DNA by adding formaldehyde directly to the culture medium to a final concentration of 1% and incubating for 10 min at 37°C. The cells were harvested after washing and lysed with SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris, pH 8.1). The liver of adult male rat (*ad libitum*) were perfused with serum free DMEM for 5 min through the portal vein, then cross-linked with 3% formaldehyde in serum free DMEM. Chromatin complexes in the supernatants were immunoprecipitated overnight with 2 μg of pre-immune serum or antibody specific for LXRα and RXRα (Santa Cruz Biotechnology, Inc.) at 4 °C with rotation. The GLUT2 promoter was used as a negative control [34]. LGK promoter-specific primers were as follow; sense, 5'-ACCAgTgTTCTgTCATC- 3'; anti-sense, 5'-ggTCTgTCTggCTgAgT-3'. A

known LXR binding site from the ABCA1 promoter was used as a positive control [35].

Adenoviral transduction

The cDNA encoding full-length human SHP into pAdTrack-CMV shuttle vector was received from Dr. Lee IK [36]. The recombinant adenovirus was amplified in 293A cells and purified using CsCl gradient centrifugation. Isolated primary hepatocytes were infected with 10 plaque-forming units per cell of Ad-SHP or Ad-null virus and incubated for 3 hr.

Small Interfering RNA experiments

The siRNAs for rat LXRα (nucleotides AUUAGCAUCCGUGGGAACAUC AGUC) and scramble (nucleotides CCUACGCCACCAAUUUCGU-dTdT) were designed. For siRNA transfection, rat primary cultured hepatocytes were incubated with DMEM containing 10%FBS and w/o antibiotics for 12hr and transfected with 200nM siRNA-scramble, siRNA-LXRa using Lipofectamine RNAiMAX following the manufacturer's protocol (Invitrogen). After 5hr, the medium containing respective ligands were changed and incubated for 24hr.

Western blot

Cell lysates were prepared using 1X passive lysis buffer (Promega, Madison, WI) containing protease inhibitors. Proteins were separated by SDS-PAGE electrophoresis and transferred to a nitrocellulose membrane (GE Healthcare, Buckinghamshire, UK). The membrane were blocked and incubated with anti-HA (Covance Inc., CA), anti-α-tubulin (Calbiochem, Darmstadt, Germany), anti-β-actin (Sigma, St. Louis, MO), anti-GFP (Cell signaling, Denver, MA), anti-SREBP-1 antibody in fresh PBS containing 1% nonfat dry milk for overnight at 4℃ with agitation. Incubated membranes were washed and treated with horseradish peroxidase-conjugated secondary antibody. Then HRP signal was detected by using enhanced chemiluminescence (ECL) substrate (GE Healthcare, Buckinghamshire, UK).

GST pull-down assay

GST and GST-RXRα proteins were expressed in *E.coli* (BL21-DE3) and conjugated with Glutathione Agarose 4B beads (Peptron, Taejeon, Korea). *In vitro* translated SHP-HA was incubated with GST or GST-RXRα in HEMG buffer (40 mM Hepes (ph 7.9, KOH), 100 mM KCl, 0.2 mM EDTA, 5 mM $MgCl_2$, 1.5 mM DTT, 0.1% NP-40, 10% glycerol) containing protease

inhibitors. Beads were washed 6 times in HEMG buffer and boiled in SDS-PAGE sample buffer analyzed by SDS-PAGE and visualized by western blotting.

III. RESULTS

LXRα activates LGK expression directly.

SREBP-1c is known to mediate the regulation of LGK gene expression by insulin. Both LGK and SREBP-1c gene expressions are known to be increased in wild type mouse liver 3 days after administration of an LXRα ligand and the effect was lost in LXR null mice [37, 38]. Oral administration of LXRα ligand for 12 hours did not increase LGK gene expression in the liver of SREBP-1c null mice, which suggested that SREBP-1c is required for LGK gene expression by LXRα ligand. However these *in vivo* results in response to an acute administration of an LXR agonist could not clearly explain the molecular interplay between LXRα and SREBP-1c in the upregulation of LGK gene expression. In order to evaluate a direct role of LXRα ligand in the regulation of LGK gene expression, primary cultured hepatocytes were treated with the LXRα ligand (T1317, 1 μM) and/or the RXRα ligand (9-CR, 1 μM) for 24 hours. The mRNA level of LGK was increased either by T1317 (11-fold) or 9-CR (35-fold) respectively (Fig. 1A). Furthermore, combined treatment of T1317 and 9-CR resulted in a synergistic increase in LGK mRNA level (80-fold). In contrast, the pattern of SREBP-1c gene expression

in response to the LXRα ligand did not match with that of LGK gene expression. SREBP-1c mRNA was increased by T1317 (80-fold) but 9-CR did not increase SREBP-1c gene expression. Additionally, the combined treatment of T1317 and 9-CR did not result in the synergistic increase in the SREBP-1c mRNA level (Fig. 1B). If LXRα dependent transcriptional induction of LGK gene is entirely mediated by SREBP-1c, the transcriptional induction pattern of LGK by the ligands of LXRα and/or RXRα would be predicted to be similar to that of SREBP-1c. The differential pattern of transcriptional induction by the ligands of LXRα and/or RXRα for LGK versus SREBP-1c suggested that LXRα might regulate LGK expression independently of SREBP-1c.

Since it is reported that T1317 could also modulate the activity of PXR, a nuclear receptor responsive to xenobiotics, we confirmed an alternate agonist of LXRα such as GW3965 in primary hepatocytes (Fig. 2). Also 9-CR is a ligand of RXRs and RARs. In order to rule out the effect of RARs, we used a LG268 compound which is RXRα selective ligands. Combined treatment of these ligands resulted in increase of LGK, FAS and SREBP-1c gene expression. FAS gene expression is known to regulate by LXRα through directly interaction with the FAS promoter as well as through indirect effects

on SREBP-1c. These results suggest that LXRα may be able to activate LGK transcription directly without the involvement of SREBP-1c.

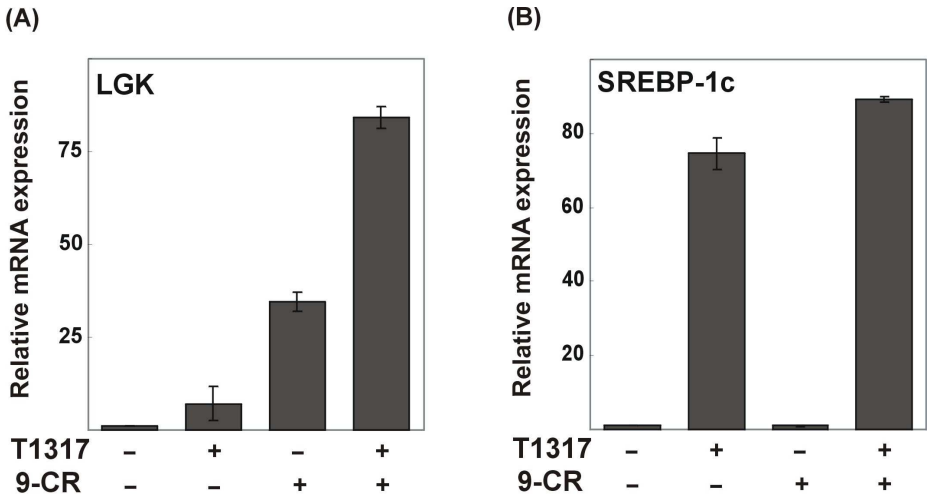


Figure 1. LXRα increases LGK mRNA level in primary cultured hepatocytes. Primary cultured hepatocytes isolated from rats were treated with T1317 (1 μM) and/or 9-CR (1 μM) for 24 hours in the presence of FBS (10 %). The mRNA levels of LGK (A), SREBP-1c (B) were quantitated by real-time PCR. Quantity of the mRNAs was normalized with respect to β-actin mRNA. Data were processed by the comparative C_T method and expressed as fold increase relative to the basal transcription level in the absence of ligands. Data are shown as the mean ± S.D. of three independent experiments.

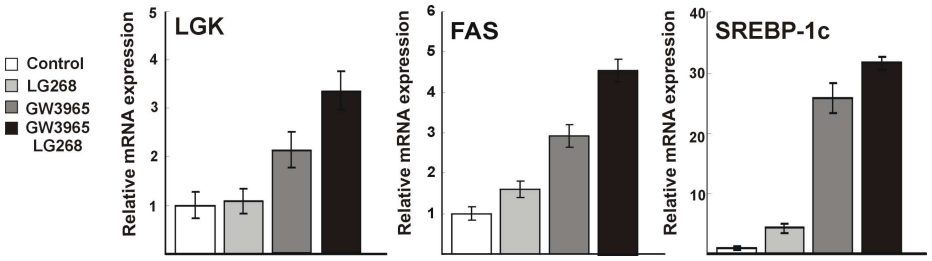


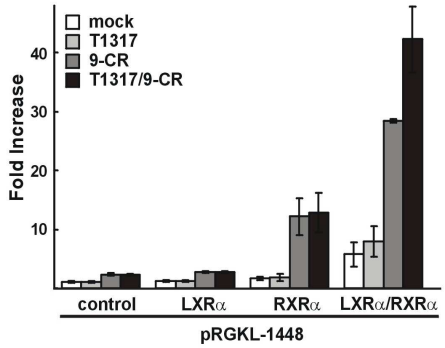
Figure 2. **LGK expression is also regulated by alternate agonist of LXRα.**

Primary cultured hepatocytes were incubated with GW3965 (5 μM) and/or LG268 (100 nM) for 24 hours in the presence of FBS (10 %). The mRNA levels of LGK, FAS and SREBP-1c were quantitated by real-time PCR. Quantity of the mRNAs was normalized with respect to β-actin mRNA. Data were processed by the comparative C_T method and expressed as fold increase relative to the basal transcription level in the absence of ligands. Data are shown as the mean ± S.D. of three independent experiments.

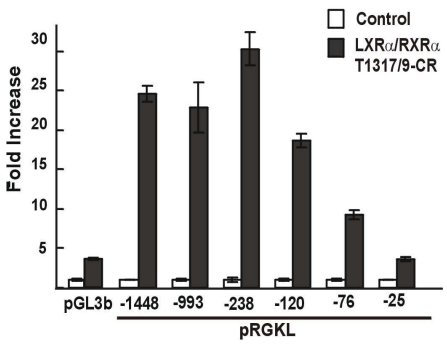
In order to explore the possibility that the LGK promoter is directly regulated by LXRα, a luciferase reporter under the control of the LGK promoter, pRGKL-1448, was transfected into the Alexander cells. The cells were cotransfected with expression vectors for LXRα and/or RXRα followed by treatment with T1317 and/or 9-CR. As shown in Figure 3A, LGK promoter activity was not activated by T1317 and/or 9-CR when LXRα and/or RXRα expression vector were not transfected. Treatment of 9-CR resulted in an increase in the LGK promoter activity in the Alexander cells with the expression of RXRα. The coexpression of LXRα and RXRα activated the LGK promoter in the absence of ligands probably due to a low concentration of endogenous oxysterol LXR ligands. The promoter activity was further increased by T1317/9-CR with the expression of LXRα and RXRα, suggesting the LGK promoter was directly activated by the LXRα/RXRα heterodimer. To localize the region responsible for the activation by LXRα, serial deletion constructs of LGK promoter-reporter were transfected into Alexander cells with the LXRα and RXRα expression vectors in the presence or absence of added T1317/9-CR (Fig. 3B). Deletion of the promoter construct down to -76 and -25 bp resulted in a significant loss of LXRα responsiveness, suggesting the presence of an LXR response element (LXRE)

in the -120/-25 bp region. Considering that a PPRE is present in -120/-76 bp region of the LGK gene promoter [27], and that PPARγ is also categorized as a permissive binding partner of RXRα, the region between -76 and -25 bp is more likely to contain an LXRE. Unfortunately, this region did not contain an easily identifiable DR4 which is the high affinity LXRE [15]. In order to find putative LXRE, five scanning mutants were prepared by introducing substitution mutations into the putative nuclear receptor half-site elements contained within the -120/-25 bp region (Fig. 3C) and the mutants were tested for responsiveness to LXRα (Fig. 3D). The PPRE mutation (m2) showed a 50% decrease in LXRα-dependent activation of LGK promoter but m4, m5 mutations retained LXRα responsiveness. Mutations at the m6, m7 sites resulted in 75% decrease in LXRα dependent activation, suggesting the presence of a functional LXRE in this region. From these data, it is assumed that the -52/-37 bp may be a LXRE and LGK could be activated by LXRα through this site and the PPRE.

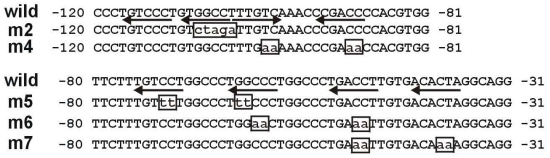
(A)


mock
T1317
9-CR
T1317/9-CR
Fold Increase
40
30
20
10
control
LXRα
RXRα
LXRα/RXRα
pRGKL-1448

(B)


Control
LXRα/RXRα
T1317/9-CR
Fold Increase
30
25
20
15
10
5
pGL3b
-1448
-993
-238
-120
-76
-25
pRGKL

(C)


wild -120 CCCTGTCCCTGTGGCCTTTGTCAAACCCGACCCCACGTGG -81
m2 -120 CCCTGTCCCTGTctagaTTGTCAAACCCGACCCCACGTGG -81
m4 -120 CCCTGTCCCTGTGGCCTTTGaaAAACCCGAaaCCACGTGG -81
wild -80 TTCTTTGTCCTGGCCCTGGCCCTGGCCCTGACCTTGTGACACTAGGCAGG -31
m5 -80 TTCTTTGTttTGGCCCTttCCCTGGCCCTGACCTTGTGACACTAGGCAGG -31
m6 -80 TTCTTTGTCCTGGCCCTGGaaCTGGCCCTGAaaTTGTGACACTAGGCAGG -31
m7 -80 TTCTTTGTCCTGGCCCTGGCCCTGGCCCTGAaaTTGTGACAaaAGGCAGG -31

(D)

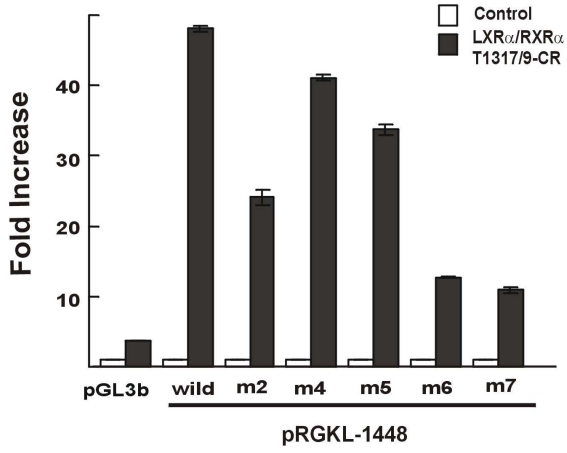

Control
LXRα/RXRα
T1317/9-CR
Fold Increase
40
30
20
10
pGL3b
wild
m2
m4
m5
m6
m7
pRGKL-1448

Figure 3. Identification of LXRE in the LGK gene promoter.

(A) Alexander cells were cotransfected with 100 ng of expression vectors for LXRα and/or RXRα and luciferase reporters under the control of rat LGK promoters (pRGKL-1448). The cells were treated with T1317 (1 μM) and/or 9-CR (1 μM) as indicated. White bar; negative control; back slash bar; T1317; slash bar; 9-CR; black bar; T1317 and 9-CR treated group. (B) 5' serial deletion constructs of the LGK gene promoter reporter were transfected into Alexander cells. The positions of mutations are shown in the Figure 3C. (D) Luciferase reporter constructs containing mutants in the LGK gene promoter were transfected into Alexander cells. The cells were cotransfected with the expression vectors for LXRα and RXRα in the presence or absence of T1317 and 9-CR. White bar; no transfection of LXRα and RXRα and no treatment with 9-CR and T1317; black bar; transfection of LXRα and RXRα and treatment with T1317 and 9-CR (B, D). Normalized luciferase activities are shown as the mean ± S.D. of three independent experiments performed in triplicate and are expressed as fold increases relative to the basal activity.

To confirm the binding of LXRα to the putative LXRE in the -52/-37 bp region of the LGK promoter, we performed electrophoretic mobility shift assays (EMSA) using *in vitro* translated LXRα-*myc*/His and RXRα-flag. As shown in Figure 4A, neither LXRα nor RXRα formed specific protein-DNA complex alone (land 3, 4). When the probes were incubated with both LXRα and RXRα, a specific shifted band was observed, indicating the binding of a LXRα /RXRα heterodimer to the LGK-LXRE (lane 5). The specificity of the shifted complex was confirmed by anti-myc antibody (lane 6). Addition of excessive amount of unlabeled competitor LXRE (50-fold) from the GLUT4 promoter completely blocked LXRα/RXRα binding to the -59/-29 bp region of LGK promoter (land 7). The direct binding of LXRα to LGK-LXRE was confirmed by chromatin immunoprecipitation (ChIP) assay in both primary cultured hepatocytes (Fig. 4B, C) and in liver (Fig. 4B, D). To characterize the specificity for LXRα antiserum, HEK293 cells were transfected with expression vector for LXRα-*myc*/His and performed immunoblot. Expression of LXRα is confirmed by LXRα antiserum and *myc* antibody (Fig. 4E).

In addition to identifying the LXRE in LGK promoter through both *in vitro* and *in vivo* approaches, we needed to confirm whether the LGK-LXRE itself could respond to LXRα/RXRα because its sequence was different from the

conventional LXRE consensus sequence. To this end, 3 copies of LGK-LXRE were subcloned in front of ptkLuc and tested its responsiveness to LXRα/RXRα. As shown in Figure 8, LGK-LXRE was activated well by LXRα/RXRα but not by PPARγ/RXRα, indicating that LGK-LXRE is functional in both the LGK promoter and the artificial promoter context. These results suggest that LXRα/RXRα binds to the LGK promoter and directly activates LGK transcription in liver.

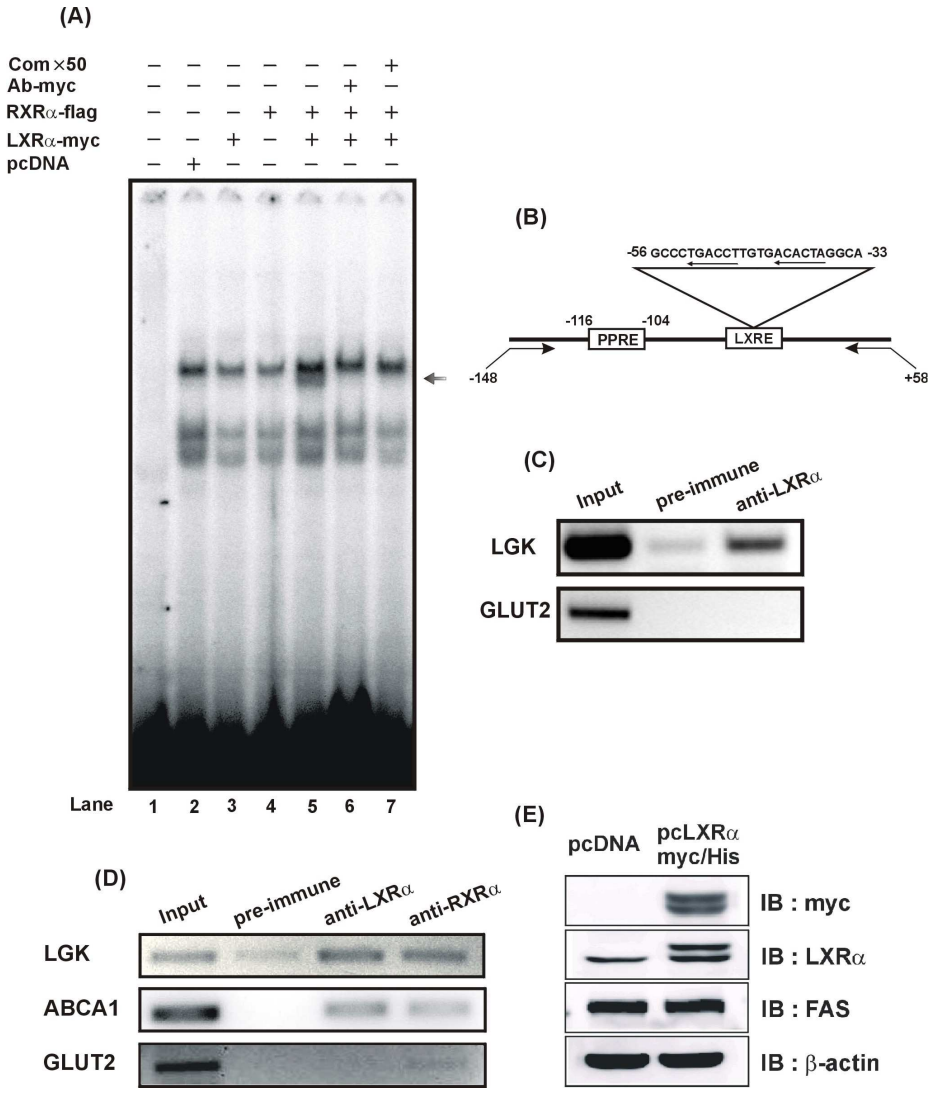

(A)
Com×50 − − − − − − +
Ab-myc − − − − − + −
RXRα-flag − − − + + + +
LXRα-myc − − + − + + +
pcDNA − + − − − − −
Lane 1 2 3 4 5 6 7
(B)
-56 GCCCTGACCTTGTGACACTAGGCA -33
-116
-104
PPRE
LXRE
-148
+58
(C)
Input
pre-immune
anti-LXRα
LGK
GLUT2
(D)
Input
pre-immune
anti-LXRα
anti-RXRα
LGK
ABCA1
GLUT2
(E)
pcDNA
pcLXRα myc/His
IB : myc
IB : LXRα
IB : FAS
IB : β-actin

Figure 4. Binding of LXRα/RXRα to the LXRE in the LGK gene promoter. (A) An electrophoretic mobility shift assay using *in vitro* translated protein from pcLXRα-*myc*/His and pcRXRα-flag expression vectors. The oligonucleotides covering the -59/-29 bp region (wild) was used as probes. ^{32}P-labeled probe was incubated with 4 μl of the *in vitro* translated protein. Unlabeled GLUT4 PPRE with 50-fold excess was used as competitor. A chromatin immunoprecipitation (ChIP) assay was adopted to confirm the binding of LXRα/RXRα to the LGK promoter in primary cultured hepatocytes (C) and liver (D). Primary cultured hepatocytes isolated from rats were treated with T1317 and 9-CR for 24 hours and then were cross-linked using formaldehyde. The liver was fixed with 3% formaldehyde by perfusing the portal vein. Chromatins were incubated with anti-LXRα antiserum and anti-RXRα antibody. DNA in the presence or in the absence of antibody was immunoprecipitated and PCR amplification of the DNA fragments was performed using primer pairs specific to the -148/+50 bp region of the rat LGK gene (B, D). (E) HEK293 cells were transfected with expression vector for pcDNA (2ug) or pcLXRα-myc/His (2ug). Protein levels were measured by immunoblot using either anti-myc or anti-LXRα serum antibodies.

LXRα activates LGK indirectly

As shown in Figure 3C, activation of LGK promoter by LXRα was compromised by a mutation in the previously characterized PPRE. Because LXRα is known to increase PPARγ expression and increase adiposity in white adipose tissue [18], it is likely this effect of LXRα be exerted through increasing PPARγ expression. In addition, LXRα is known to increase the expression of SREBP-1c which in turn binds to two SREs in LGK promoter and increases LGK gene expression in response to insulin. Together, PPARγ and SREBP-1c are likely to be involved in the regulation of the LGK promoter by LXRα. To explore these interrelationships further, we prepared LGK promoter reporter constructs with various combinations of mutations in the SRE, PPRE and LXRE (Fig. 5). The mutated LGK promoter constructs were cotransfected into Alexander cells with expression vectors for LXRα/RXRα and treated with their respective ligands. Mutation of the PPRE (m2) or the LXRE (m7) resulted in 60% decrease in LXRα-dependent activation, whereas the SRE mutations (mab) did not cause any decrease in the LXRα-dependent activation. Introduction of SRE mutations into the PPRE mutant (m2ab) or LXRE mutant (m7ab) did not result in a further decrease in LXRα-dependent activation of LGK promoter when compared to PPRE mutant (m2) or LXRE

mutant (m7). Double mutations in LXRE and PPRE (m27) and triple mutations (m27ab) caused the most significant decrease in the LXRα-dependent activation of LGK promoter. These results indicate that LXRα-dependent activation of the LGK promoter in Alexander cells is partly through the direct activation of the LXRE and partly by indirect activation through the PPRE.

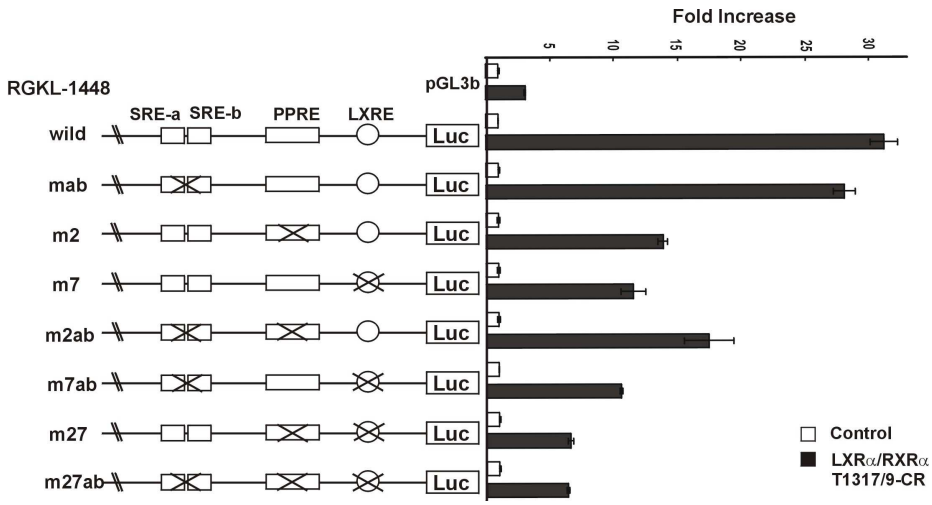


Figure 5. Functional relationships among SREBP-1c, PPARγ and LXRα in the activation of the LGK gene promoter. Luciferase reporter constructs of the LGK promoter and their mutants were transfected into Alexander cells. The positions of the mutations are shown. The cells were cotransfected with the expression vectors for LXRα and RXRα in the presence or absence of T1317 (1 μM) and/or 9-CR (1 μM). White bar; no transfection of LXRα and RXRα and no treatment with 9-CR and T1317; black bar; transfection of LXRα and RXRα and treatment with T1317 and 9-CR. Normalized luciferase activities are shown as the mean ± S.D. of three independent experiments performed in triplicate and are expressed as fold increases relative to the basal activity.

In an attempt to confirm the effects of LXRα in connection with SREBP-1c, primary hepatocytes were treated with T1317/9-CR for 24 hours in the presence or absence of insulin As shown in Figure 6A, T1317/9-CR increased LGK mRNA (7-fold) and insulin increased LGK mRNA level (6-fold). The combination of T1317/9-CR/insulin increased LGK mRNA level synergistically (22-fold). It is reported that LXRα induced-full induction of the mature and transcriptionally active form of SREBP-1c protein requires insulin [39]. These results suggested that LXRα may directly activate LGK gene expression in the absence of insulin. Consistent with this prediction, the increase of LGK mRNA by T1317/9-CR in the presence of insulin could be explained by following effects: (a) LXRα directly upregulates LGK gene transcription, (b) LXRα increases expression of the precursor form of SREBP-1c which is cleaved in the presence of insulin and entered nucleus, (c) increased nuclear SREBP-1c further stimulates LGK gene expression. when primary cultured hepatocytes were treated with adenoviral-SREBP-1 DN(Ad-ADDN) in the presence or absence of insulin, the ADDN did not affect either LXRα mediated-LGK or FAS mRNA level in absence of insulin. This observation suggests that LGK gene expression is directly induced by LXRα without affecting SREBP-1c. In addition, synergistic increase of LGK

mRNA expression in the presence of insulin is mediated by SREBP-1c. Also, expression of FAS was not affected by adenoviral-SREBP-1 DN in the absence of insulin and decreased by effect of insulin-mediated SREBP-1c in the presence of insulin.

We further examined the effect of SREBP-1c and LXRα on LGK expression. LXRα-induced LGK promoter activity was further activated by SREBP-1c expression and mutation of SREBP binding site (m7) resulted in complete loss of additive activation. Taken together, these results indicated that LXRα and SREBP-1c additively regulated the LGK gene promoter.

In contrast to LGK mRNA levels, T1317/9-CR did not affect the transcription of LXRα and PPARγ (data not shown). These results suggest that upregulation of LGK gene expression by LXRα and PPARγ occurs not by increasing their levels but by increasing their transcriptional stimulation activity.

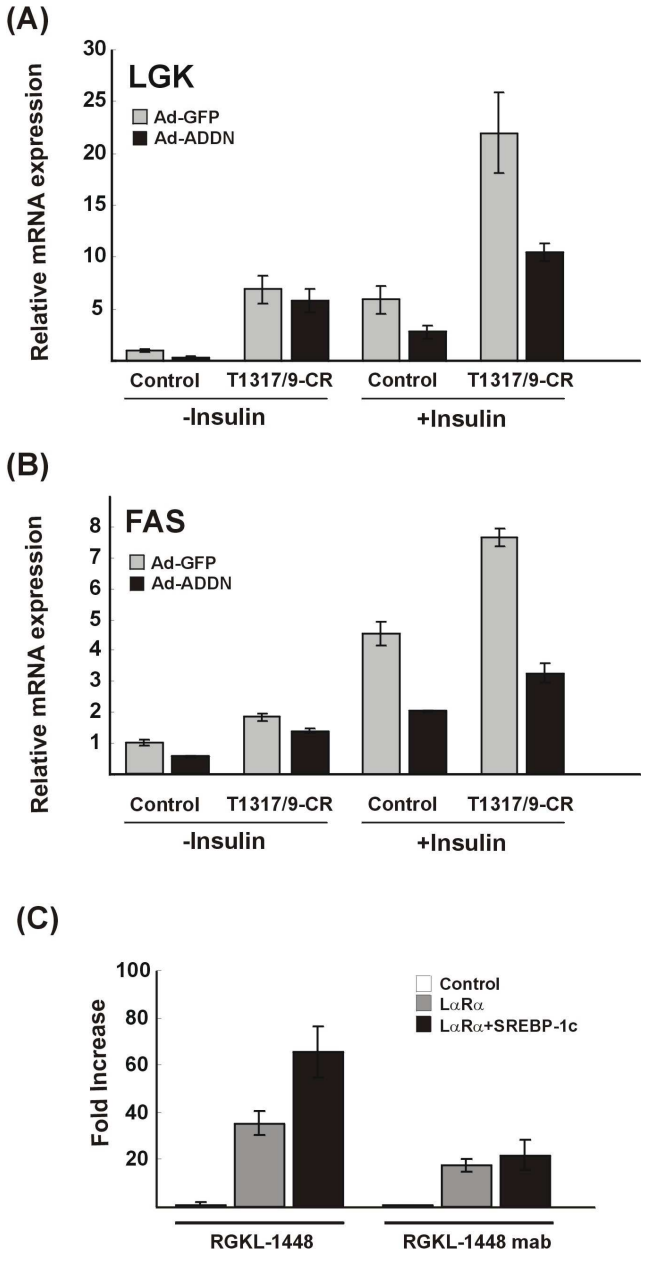

(A)
LGK
Ad-GFP
Ad-ADDN
Relative mRNA expression
30
25
20
15
10
5
Control
T1317/9-CR
Control
T1317/9-CR
-Insulin
+Insulin
(B)
FAS
Ad-GFP
Ad-ADDN
Relative mRNA expression
8
7
6
5
4
3
2
1
Control
T1317/9-CR
Control
T1317/9-CR
-Insulin
+Insulin
(C)
Fold Increase
100
80
60
40
20
Control
LαRα
LαRα+SREBP-1c
RGKL-1448
RGKL-1448 mab

Figure 6. Effect of LXRα and insulin on the LGK and FAS mRNA levels and promoter activity. Primary cultured hepatocytes were isolated from rat liver and infected with Ad-GFP (10MOI) or Ad-ADDN (10MOI) for 3hr. Cells were treated with insulin (100 nM) and/or T1317 (1 μM) and 9-CR (1 μM) for 24 hours in the absence of insulin as indicated. The mRNA levels of LGK (A) and FAS (B) were quantitated by real time PCR. Quantity of the mRNAs was normalized with respect to β-actin mRNA. Data were processed by the comparative C_T method and expressed as fold increase relative to the basal transcription level in the absence of ligands. (C) Alexander cells were transiently transfected with the wild type -1448 LGK promoter reporter of construct containing specific mutation of SRE binding site (-1448mab). Expression vector for LXRα, RXRα and SREBP-1c were included as indicated. Cells were treated with control or 1uM T1317/1uM 9-CR for 24hr. Data are shown as the mean ± S.D. of three independent experiments.

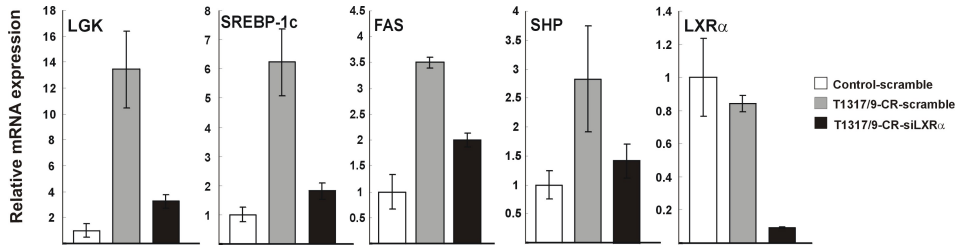


Figure 7. Effect of LXRα siRNA on the T1317/9-CR induced LGK, SREBP-1c, FAS and SHP gene expression in primary hepatocytes.

Primary hepatocytes were isolated from rat and incubated for attach. The cells were transfected with 200nM siRNA-scramble (scramble) or siRNA-LXRa (siLXRα) using Lipofectamine RNAiMAX. After 5hr, the media were changed containing with 1uM T1317/1uM 9-CR ligands and the cells were incubated for 24hr. The mRNA levels of LGK, SREBP-1c, FAS, SHP and LXRα were quantitated by real time PCR. Quantity of the mRNAs was normalized with respect to β-actin mRNA. Data were processed by the comparative C_T method and expressed as fold increase relative to the basal transcription level in the absence of ligands.

Most of the activity attributed to RXR/LXR appears to be mediated by 9-CR, not T1317. And RXR ligands can activate other heterodimers. To elucidate the LXRα is essential for ligand induced-LGK gene expression, we used a siRNA-LXRα (siLXRα) for knockdown of LXRα in primary hepatocytes. T1317/9-CR activates the gene expression of LGK and increased LGK mRNA expression is abolished by treatment of siLXRα. Known target of LXRα such as SREBP-1c, FAS and SHP were also decreased by siLXRα. These data suggest that LXRα is required for T1317/9-CR induced-LGK gene expression.

To test our hypothesis for indirect activation of the PPRE in the LGK promoter by LXRα, 3 copies of each of the LGK-PPRE and LGK-LXRE were subcloned in front of a minimal TK promoter in a luciferase reporter and the resulting constructs were tested for responsiveness to LXRα/RXRα, PPARγ/RXRα (Fig. 8). The LGK-PPRE luciferase reporter was activated both by LXRα/RXRα and PPARγ/RXRα. However the LGK-LXRE was activated only by LXRα/RXRα. Expression of the PPARγ and LXRα genes were not increased by the LXRα ligand, the activation of the PPRE by LXRα/RXRα suggested that LXRα indirectly activates the LGK-PPRE by increasing transcriptional activity of PPARγ, possibly through stimulating production of a PPARγ ligand.

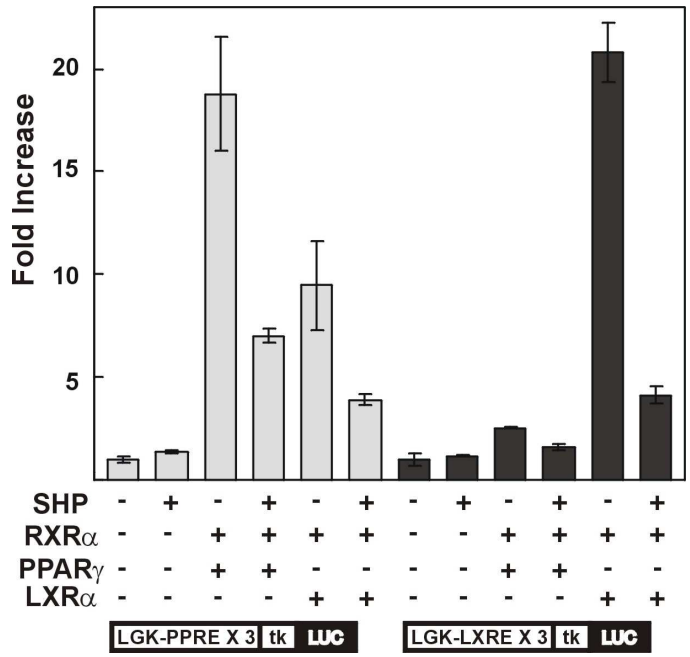


Figure 8. LXRα activates PPRE by increasing the transcriptional activity of the PPARγ. Alexander cells were cotransfected with 100 ng of expression vectors for LXRα, PPARγ, RXRα and the luciferase reporter containing the 3 copies of LGK-PPRE or LGK-LXRE in front of a thymidine kinase minimal promoter as indicated. The LGK-PPRE construct (includes the -119/-98 of the LGK promoter) and LGK-LXRE construct (-56/-32 region) were transfected with LXRα, PPARγ and/or RXRα into Alexander cells. The cells were treated with their ligands as indicated. Normalized luciferase activities are shown as the mean ± S.D. of three independent experiments performed in triplicate.

SHP functions as a negative modulator of LGK gene transcription

SHP interacts with various transcriptional factors and represses their transcriptional activity either by competing with coactivators or recruiting corepressors [21, 40]. SHP is also known to interact with LXRα and RXRα and inhibit the transcriptional activity of these receptors [25, 40]. In contrast to LXRα and RXRα, SHP was reported to augment PPARγ transcriptional activity by competing with the binding of a corepressor [26]. In addition, SHP null mice showed increased LGK expression in liver [24]. Because of the opposite effects of SHP on LXRα and PPARγ, these observations led us to explore the role of SHP in transcriptional regulation of LGK these two nuclear receptors.

To examine the effect of SHP on LGK, the LGK promoter reporter construct (pRGKL-1448) was transfected to Alexander cells and an expression vector for SHP was added in addition to vectors for LXRα or PPARγ. As shown in Figure 9A, LXRα dependent activation of LGK promoter was decreased by SHP in a dose dependent manner. When equal amount of SHP and LXRα were transfected, most of the LXRα dependent transcriptional activation was abolished. SHP also inhibited PPARγ dependent activation of LGK promoter (Fig. 9B). The SHP dependent inhibition of LGK promoter activity was further confirmed using the LGK-PPRE and LGK-

LXRE synthetic reporter promoter constructs as well (Fig. 8).

We also showed that SHP inhibited the PPARγ or LXRα dependent stimulation of the endogenous LGK gene in primary cultured hepatocytes using an adenovirus expressing SHP (Fig. 9C). It is interesting to note that the precursor form of SREBP-1c was increased by T1317/9-CR. However, the SREBP-1 protein level was not altered by SHP transduction (Fig. 9C), suggesting that SHP could inhibit LXRα dependent LGK gene expression without affecting SREBP-1c gene expression. The SHP adenovirus also inhibited the PPARγ mediated increase in LGK gene expression (Fig. 9D). These results suggest that SHP functions as a negative modulator of the PPARγ and LXRα dependent induction of LGK gene transcription.

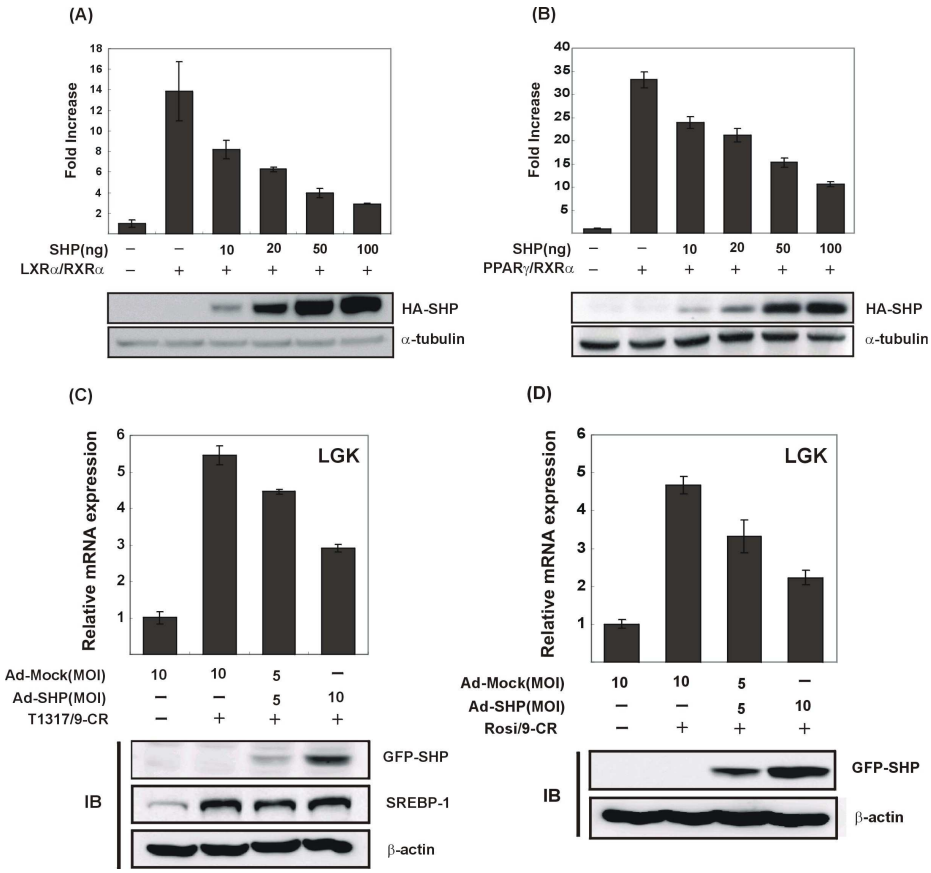


Figure 9. SHP inhibits LXRα and PPARγ-mediated LGK promoter activity.

Alexander cells were cotransfected with expression vectors for LXRα (100 ng) , PPARγ (100 ng), or RXRα (100 ng), with increasing amount of SHP expression vector and LGK promoter-luciferase reporter (pRGKL-1448). Cells were treated with their respective ligands appropriately. The expression of SHP protein is confirmed by immunoblotting. Normalized luciferase

activities are shown as mean ± S.D. of three independent experiments in a triplicate and are expressed as fold increase relative to the basal activity. The expression of HA-SHP protein was validated by immunoblotting (A, B). Rat primary cultured hepatocytes were transduced by adenoviral expression of SHP. After 3hours, T1317 (1 uM)/9-CR (1 uM) and rosiglitazone (1 uM)/9-CR(1 uM) were treated for 24hours. The transcription levels of LGK were quantitated by real-time PCR. The expression of GFP-SHP was validated by immunoblotting (C, D). HA: hemagglutinin ; GFP: green fluorescent protein.

These data are contradictory to the previous report that SHP augmented the transcriptional activity of PPARγ in adipocytes [26, 41]. In order to explore a molecular mechanism for how SHP inhibits PPARγ dependent activation in hepatocytes, we performed a mammalian two hybrid assay with minor modifications using a luciferase reporter that has upstream activating sequence (UAS) in front of the TK minimal promoter (Fig. 10). We employed a system utilizing a GAL4-PPARγ fusion and SHP fused with or without the VP16 activation domain or the reciprocal combination of fusion proteins. As shown in Figure 10A, expression of the Gal4-PPARγ fusion protein increased luciferase activity in the presence of rosiglitazone (1 μM). However, neither SHP nor VP16-SHP affected Gal4-PPARγ activity. We further confirmed the interaction of PPARγ and SHP in the reciprocal combination. Gal4-SHP expression decreased luciferase activity because of the intrinsic repression function of SHP. Coexpression of Gal4-SHP and VP16-PPARγ did not stimulate the luciferase activity from the repressed state which was observed in Gal4-SHP alone. This result suggested that PPARγ did not interact with SHP. In contrast, SHP did repress activation mediated by the Gal4-RXRα fusion protein activator (Fig. 10B).

When we checked the interaction between RXRα and SHP with the

reciprocal combination, Gal4-SHP decreased luciferase activity and coexpression of VP16-RXRα with Gal4-SHP stimulated the luciferase activity from the repressed state. The interaction between RXRα and SHP was further confirmed by GST pull-down assays using *in vitro* translated HA-SHP and GST-RXRα fusion protein (Fig. 10C). These results indicate that SHP functions as a negative modulator of the PPARγ and LXRα mediated LGK gene transcription by interacting RXRα.

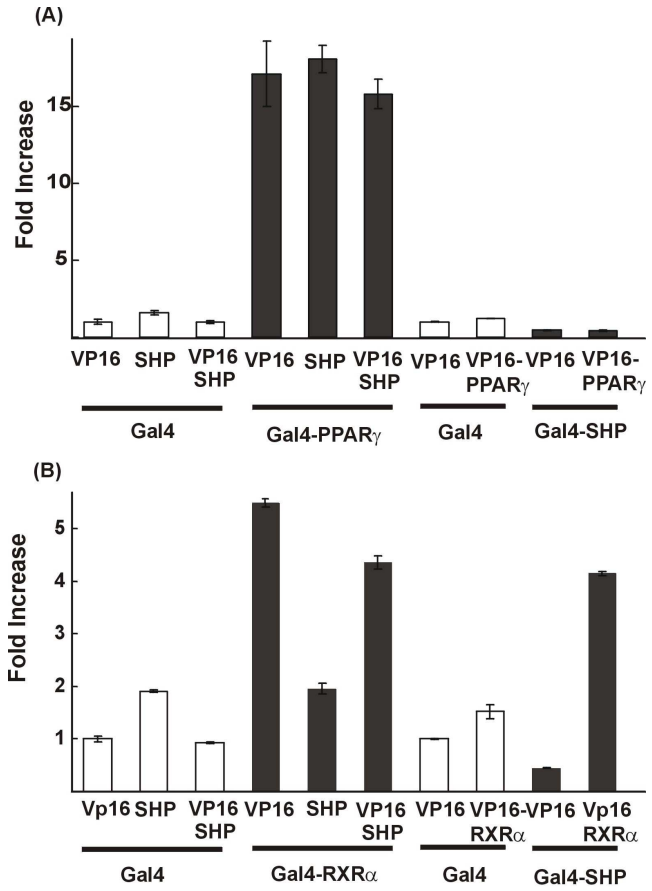

(A)
Fold Increase
15
10
5
VP16
SHP
VP16 SHP
VP16
SHP
VP16 SHP
VP16
VP16-PPARγ
VP16
VP16-PPARγ
Gal4
Gal4-PPARγ
Gal4
Gal4-SHP
(B)
Fold Increase
5
4
3
2
1
Vp16
SHP
VP16 SHP
VP16
SHP
VP16 SHP
VP16
VP16-RXRα
VP16
Vp16 RXRα
Gal4
Gal4-RXRα
Gal4
Gal4-SHP
(C)
20% Input
GST
GST RXRα
Bead
34 kDa
Anti-HA
kDa
GST
GST RXRα
95
72
55
43
34
26

Figure 10. SHP interacts with RXRα but not with PPARγ .

Alexander cells were cotransfected with expression vectors for the Gal4DBD (100 ng) or fusion proteins Gal4DBD-PPARγ (100 ng), Gal4DBD-RXRα (100 ng), Gal4DBD-SHP (100 ng), VP16 (100 ng) or VP16-PPARγ (100 ng), VP16-RXRα (100 ng), VP16-SHP (100 ng) and the luciferase reporter construct (pUAS) containing 3 copies of Gal4 binding site in front of TK minimal promoter. The cells were treated with rosiglitazone (1 μM) for PPARγ or 9-CR (1 μM) for RXRα. Normalized luciferase activities are shown as mean ± S.D. of three independent experiments in a triplicate and are expressed as fold increase relative to the basal activity (A, B). In vitro translated SHP-HA protein was incubated with GST, GST-RXRα fusion protein bound to glutathione-Q Sepharose beads. Protein interaction were detected by immunoblotting using anti-HA antibody. The quantity of GST and GST-RXRα fusion protein were confirmed by Coomassie brilliant blue staining (C). HA: hemagglutinin

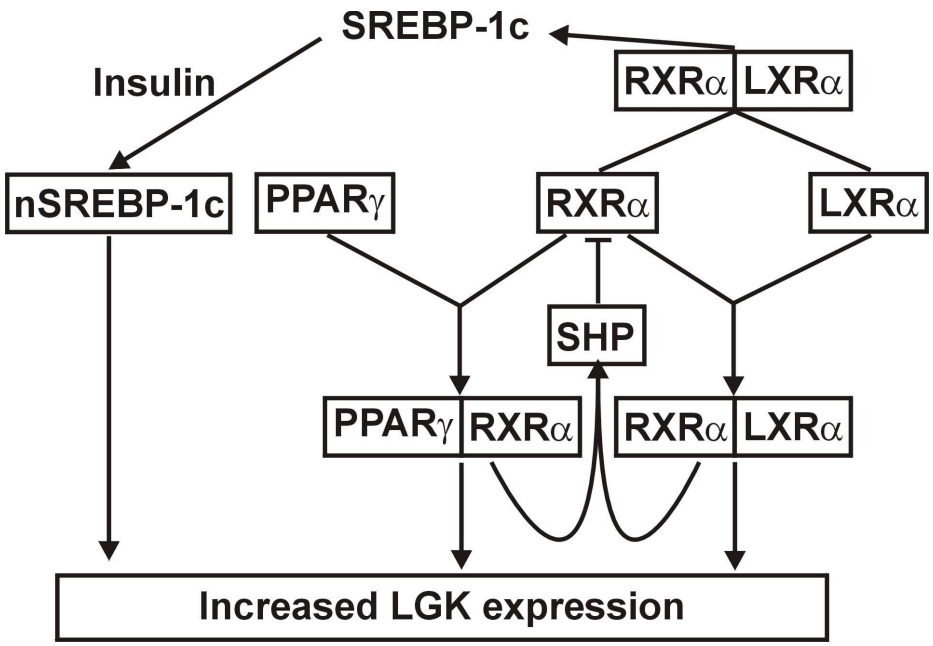


Figure 11. A model showing interrelationship between LXRα, SREBP-1c, PPARγ and SHP in the transcriptional regulation of LGK

LXRα/RXRα is shown to bind and activate the LXRE in the LGK gene promoter. Reciprocally, the expression of LGK gene was upregulated by LXRα through either inducing SREBP-1c gene or increasing the transcriptional activity of PPARγ. In addition, SHP is also upregulated by LXRα/RXRα and PPARγ/RXRα resulting in a decrease in the repression of LGK promoter activities by binding to RXRα.

IV. DISCUSSION

Intracellular glucose-6-phosphate is the primary physiologic stimulus for the hepatic glucose metabolism as it is a substrate for both glycolysis and glycogen synthesis [42] and small changes in the expression of the enzyme LGK which produces glucose-6-phosphate from glucose can have a profound impact on the blood glucose concentration [1, 2, 43]. Here we present data that LXRα plays an orchestrated role in the regulation of LGK expression. We have identified an LXRE in the LGK promoter and shown that LXRα activates LGK expression directly through binding to its promoter. We have also shown that LXRα activates the LGK promoter indirectly through a mechanism requiring PPARγ and SREBP-1c. In addition, we show that SHP functions as a negative modulator of LGK transcription by inhibiting the transcriptional activity of LXRα and RXRα.

LXRα has an insulin like effect on hepatic carbohydrate metabolism by stimulating genes involved in glucose storage and inhibiting gluconeogenesis, which would be consistent with a role in promoting energy storage in a model of diet-induced obesity and insulin resistance [38]. Thus LXRα has an important role in the coordination of lipid and glucose metabolism, which suggests that

LXRα ligands may have therapeutic potential through the modulation of glucose homeostasis. LXRα is known to activate SREBP-1c expression and thereby increase the activity of genes involved in glycolysis and lipogenesis [14, 44]. However there is still question about the relationship between SREBP-1c and LXRα and whether LXRα is involved directly in the physiologic effects of insulin or whether LXRα's role is solely to activate SREBP-1c.

Insulin is known to increase LXRα dependent SREBP-1c expression by producing endogenous LXRα ligands [44]. Recently Hegarty *et. al.* showed that conversion of the membrane bound SREBP-1c precursor into the mature soluble transcription factor is enhanced after acute exposure to insulin [39] and LXRα increases LGK expression in primary cultured hepatocytes by increasing SREBP-1c gene expression in the presence of insulin. Administration of the LXRα ligand GW3965 for 3 days increased LGK expression in mouse liver. In addition, treatment of mice with another synthetic LXR ligand T1317 for 12 hours did not increase LGK gene expression in the SREBP-1c null mouse [38, 45]. These data suggested that LGK induction by LXRα is mediated by SREBP-1c. But there is a caveat to be considered. When Liang *et. al.* tested GK gene expression in the liver of the SREBP-1c null mouse, they treated mice with GW3695 instead of T1317 only

for 12 hours in a chow diet. This treatment protocol seemed to be less effective than 3 days' gavage feeding of T1317, which potentially makes it more difficult to observe changes in gene expression which is not strongly activated by LXRα. In addition to this, we used rat LGK gene and rat primary cultured hepatocytes instead of mouse. Thus, there might be a difference between species in the regulation mechanism of LGK expression by LXRα. In the present study, we identified LXRE in LGK promoter and showed that the *cis*-element is functional in both promoter construct and artificial promoter context and confirmed the binding of LXRα/RXRα by EMSA and ChIP assay. We also demonstrated that LXRα ligand, T1317, increased LGK gene expression in primary cultured hepatocytes independent of insulin. If stimulation of LGK gene expression by LXRα were entirely dependent on of the prior activation of SREBP-1c, then the change in LGK expression pattern by T1317 and 9-CR would be comparable to the activation provided by insulin alone but there is enhanced stimulation of LGK expression by the combination of insulin and the nuclear receptor agonists.

We have also observed that LXRα activates the LGK promoter through a PPRE. This is likely to be indirect, because it has been reported that LXRα increases the production of endogenous PPARγ agonists through the

upregulation of SREBP-1c gene [46]. In these studies, a role for ligand activation of RXRα, a permissive partner of LXRα, cannot be ruled out. However, overexpression of RXRα alone did not activate LGK-PPRE in artificial promoter context (data not shown). Based on all of our experiments, we propose a novel mechanism of activation for LGK by LXRα which involves both SREBP-1c and PPARγ.

Finally, we also showed that SHP inhibited the transcriptional activation of the LGK promoter by LXRα and PPARγ. We also showed that adenoviral expression of SHP significantly decreased LGK expression in the primary hepatocytes. Our results suggest that the effects of SHP are through its interaction with RXRα resulting in the decrease in LGK promoter activity. Importantly, these data are consistent with the observation that in the liver of the SHP-/- mice, mRNA levels of glycolytic enzymes, such as glucokinase (GK) and pyruvate kinase (PK), were increased.

To explain the interactive mechanism suggested by our study, we provide a model summarizing the orchestrated role of PPARγ, LXRα, SREBP-1c and the negative modulator SHP in the regulation of LGK gene transcription (Fig. 11). In this model, LXRα activates the expression of LGK and SREBP-1c by directly binding to a *cis*-element in their promoters. Nuclear SREBP-1c

increases the expression of LGK and the positive regulatory circuit between LXRα and SREBP-1c makes it possible that treatment with an LXRα ligand and insulin results in synergistic stimulation of LGK gene expression. At the same time we propose that LXRα increases LGK gene expression by increasing transcriptional activity of PPARγ, possibly through increased production of an endogenous PPARγ ligand. LXRα and PPARγ increase the transcription of SHP gene, which in turn represses the transcription of LGK gene by LXRα and PPARγ by interacting with RXRα.

V. CONCLUSION

When plasma glucose concentration is excess, LGK acts as a glucose sensor to phosphorylate the glucose. Converted glucose-6-phosphate in liver goes into glycogen synthesis or catabolism. Therefore LGK is essential for maintaining the blood glucose level and metabolic function. It is reported that LGK gene is decreased in the liver of LXR null mice and increased in mouse after 3 days administration of LXRα agonist. However, precise mechanism of LGK promoter regulation has not been elucidated.

In this study, we demonstrate that orchestrated interplay of LXRα, SREBP-1c and PPARγ in the regulation of LGK transcription. An LXRα response element was identified in the LGK promoter, which is directly regulated by LXRα. Also, LXRα indirectly increases LGK gene expression by inducing the SREBP-1c gene expression and increasing the transcriptional activity of PPARγ. In addition, to investigate a molecular mechanism for how coregulator controls PPARγ/LXRα dependent activation of LGK gene expression, we test the role of SHP in LXR mediated LGK gene transcription.

We confirm that LXRα/PPARγ-dependent LGK gene expression is down regulated by SHP by directly interacting with RXRα, which is common

partner of LXRα and PPARγ. Taken together, LGK gene expression is activated by LXRα by interplay with PPARγ, SREBP-1c. Also, SHP functions as a negative modulator of LXRα mediated LGK transcription.

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ABSTRACT (IN KOREAN)

간장에서 glucokinase 유전자 전사조절에 LXRα, SREBP-1c, PPARγ 와 SHP의 상호조절 기전

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김 태 현

간장에서의 glucokinase는 혈액 내의 포도당의 농도를 유지하고 조절할 수 있는 역할을 수행한다. Glucokinase는 SREBP-1c라는 전사조절인자를 매개로 하여 insulin에 의해 유전자 발현이 유도된다. LXRα라는 전사조절 인자가 소실된 mice에서 glucokinase 유전자 발현이 감소되어 나타나기 때문에, 본 연구에서 glucokinase 유전자가 LXRα에 의해 직접적으로 조절 될 수 있는지 확인하였다. 이러한 glucokinase를 조절 한다고 알려진 다른 전사인자들과의 상호작용이 존재하는지를 확인 하였다. 본 연구에서 LXRα에 의해 glucokinase의

전사체가 증가하는 것을 rat primary hepatocytes를 분리하여 확인 하였고, glucokinase 유전자 promoter에 LXRE 가 존재함을 EMSA, ChIP assay를 통하여 밝혔다. Glucokinase의 유전자 조절은 다양한 방법을 통해 일어나게 되는데 LXRα에 의해 증가된 SREBP-1c가 insulin이 있는 상태에서 핵 안으로 들어가 활성화 되어 glucokinase유전자를 증가시키고, 또한 LXRα에 의해 PPARγ의 전사활성이 증가되어 PPARγ를 통해 glucokinase 유전자를 증가시킨다.이러한 glucokinase 유전자 전사 활성은 SHP에 의해 감소하는 효과를 보이는데, LXRα, PPARγ와 heterodimer를 이루는 RXRα와 interaction을 통하여 증가된 glucokinase 유전자 전사활성을 낮추게 된다. 이러한 결과를 통해 간장에서 glucokinase 유전자를 조절하는 LXRα은 직접적으로 promoter에 작용해서 증가시키기도 하며 SREBP-1c와 PPARγ를 통해서 증가시키고, 이러한 glucokinase 유전자 전사활성의 증가는 SHP를 통해 감소시킬 수 있는 점을 제시하고 있다.

핵심되는 말: Glucokinase, LXRα, SREBP-1c, PPARγ, SHP, Glucose metabolism