Liver type glucokinase can be activated by LXRα

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Directed by Professor **Yong-Ho Ahn**

The Master's Thesis Submitted to the Department of Medical Science, the Graduate School of Yonsei University in the partial fulfillment of the requirements for the degree of Master of Medical Science

Tae-Hyun Kim

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Tae-Hyun Kim

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Abstract

Liver type glucokinase can be activated by LXRα

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Liver type glucokinase (LGK) promotes hepatic glucose utilization and plays an essential role in the glycolysis and glycogenesis pathways. The regulation of liver type glucokinase (LGK) is important for the liver in sensing glucose.

The nuclear receptor, liver X receptor (LXR)α and LXRβ have been implicated in gene expression linked to lipid and cholesterol metabolism. Here, we present data to suggest that the LXR plays a role in glucose homeostasis through the expression of LGK.

We observed that activation of LXRα with TO-901317 lead to the induction of

hepatic glucokinase expression. And the LGK promoter is a direct target for the LXR/RXR heterodimer. We were able to localize a LXRE in the -52/-37 region of the rat LGK gene. Also, LXRα induce the expression of the LGK gene via direct activation and via sterol regulatory element binding proterin 1-c (SREBP-1c) and peroxisome proliferator-activated receptor-γ (PPARγ) stimulated by LXRα ligand. Furthermore, LXRα ligand TO-901317 lowers plasma glucose level in *ob/ob* mice. These results indicate that LXRα can directly and indirectly activate LGK expression in liver and encourage to improving glucose homeostasis.

Key Words : Liver type glucokinase (LGK), liver X receptor (LXR), glucose homeostasis, transcriptional regulation

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Ⅰ. Introduction

When the blood glucose concentration is high, as it is after a meal rich in carbohydrates, excess glucose is transported into hepatocytes, where glucokinase converts it to glucose-6-phosphate.

Glucokinase provides the capacity for glucose phosphorylation and is a rate-limiting factor in the induction of glycolysis and glycogen synthesis[1]. Because the K_m of GK is considerably higher than that of normal blood concentrations and GK is not subjected to allosteric regulation by the product, the rate of glucose phosphorylation is

proportional to the blood glucose level. Therefore, GK is essential for the liver in sensing glucose and maintaining the metabolic function[2, 3].

GK is acutely regulated by the GK regulatory protein (GKRP), which is mainly localized in the nucleus[4]. At low glucose concentration, GKRP is associated with GK in the nucleus. Acute glucose challenge induces dissociation of the GK-GKRP complex, then GK is translocated into the cytoplasm resulting in increased GK activity[5]. The activity of GK is also controlled at the transcription and/or translation levels. The GK gene contains two distinctive promoters, initially believe to be specific for pancreatic β-cells and hepatocytes. Of these two promoters, the downstream promoter regulates liver type GK (LGK) expression[6]. However, these seem to be involved in differential expression in the variety of cells where GK is expressed.

LXRα is a nuclear hormone receptor with high hepatic expression that is activated by oxysterols, such as 22(R)-hydroxycholesterol, 24, 25(S)-epoxycholesterol and 27-hydroxycholesterol, and by the synthetic compound TO-901317 and GW3965[7]. LXR heterodimerizes with RXRα then bind directly to the DNA that contains two direct repeat sequences (AGGTCA) separated by four nucleotide (DR+4). The expression of LXRα is restricted, with highest levels in the liver and lower but significant levels in kidney, intestine, spleen. Whereas LXRβ expression is more widespread[8, 9].

LXRα is essential for the regulation of cholesterol homeostasis. LXRα knock out mice showed rapid accumulation of cholesterol in liver[10]. LXRα is known to increase nuclear SREBP-1c expression in the presence of insulin[11], thereby increase glycolysis

and lipogenesis in liver[12]. It has been reported that the activation of LXR inhibited the expression of hepatic gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6 phosphatase (G6P)[13]. However, the direct role of LXRα in the glycolytic enzyme genes are not studied so far. Here, we have observed the effect of LXRα ligand on the liver type glucokinase (LGK). We have attempted to identify and characterize the *cis* element, LXRE, in the LGK promoter. Also we have observed that the LXRα mediated activation of GK is related to PPARγ and SREBP-1c. Finally we demonstrated that a treatment of the LXRα ligand significantly improved glucose tolerance in *ob/ob* mice through LGK gene expression.

Ⅱ. Materials and Methods

1. ***Cell culture and Transient transfection***

Alexander cells obtained from ATCC were cared for transfection assay. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/㎖ penicillin and 100 ㎍/㎖ streptomycin. Alexander cells were plated in tissue culture plates. After a 24h attachment period, cells were transfected with the indicated plasmids by using Lipofectamine Plus reagent (Invitrogen) according to the manufacturer's protocols. Briefly, the plasmid DNA and 4 ㎕ of PLUS reagent were mixed in 100㎕ of Opti-MEM (GIBCO) and then added 100㎕ of Opti-MEM containing 2㎕ of Lipofectamine reagent were added. The cells were washed with PBS and supplied with serum free Opti-MEM. The cells were transfected for 3hr with the plasmid and grown in DMEM supplemented with FBS. After 24hr, the cells were treated with LXRα ligand TO-901317 (1*u*M) and RXRα ligand 9 *cis*-retinoic acid (1*u*M). The cells were harvested and lysed by Reporter lysis buffer (Promega). Luciferase activities were measured using 50㎕ Luciferase assay reagent (promega). For β-galactosidase assay, the hydrolysis of O-nitrophenol-B-D-galactopyranoside (Sigma Aldrich, St. Louis, MO) at 37˚C was measured at 420㎚. Luciferase activity in relative light units was normalized to β-galactosidase activity for each sample.

2. ***Plasmids***

The rat LGK promoter-reporter construct pRGKL-1448 which containing the -1448/+127 region of the rat LGK gene was prepared and transfected into Alexander cells . To determine which *cis*-acting elements is involved in its regulation, we performed 5' deletion analysis of the glucokinase promoter. The LXRE truncated promoter reporter T-52/-37, the PPRE truncated promoter reporter T-120/-77, T-52/-37 was constructed by LXRE truncated primer. pRGKL-1448mab (SREa, SREb regions mutant), pRGKL-1448m2 (PPRE mutant) was described early[14, 15]. pRGKL-1448m7 (LXRE mutant), pRGKL-1448mabm2, pRGKL-1448mabm7, pRGKL-1448m2m7, pRGKL-1448mabm2m7 were produced by introducing a substitution mutation into pRGKL-1448 using the site directed mutagenesis. Expression plasmids of LXRα/β were gifts from David J. Mangelsdorf.

3. ***RNA Extraction and Northern Blot***

Total RNA was extracted from primary hepatocyte using TRIzol Reagent (Invitrogen, USA). The sample of 20μg of RNA was denatured with RNA sample loading buffer (20 mM MOPS, pH 7.0, 2 mM sodium acetate, 1mM EDTA, 8 % (v/v)formaldehyde, 50 % (v/v) formamide) and subjected to formaldehyde denatured agarose electrophoresis and transferred to nytran membrane (Schleicher & Schuell, Germany). The cDNA fragment of rat liver GK gene was labeled with 〔α-^{32}P〕dCTP using Rediprime Labeling Kit (Amersham Pharmacia Biotech). After hybridization, the

membrane was washed twice with high salt washing buffer (2× SSC, 0.1% SDS) at room temperature for 15 min followed by low salt washing buffer (0.2 × SSC , 0.1% SDS) at 65 ˚C for 15 min. The membrane was exposed to Kodak BioMAX film with intensifying screen at -70 ˚C.

4. *Electrophoretic Mobility Shift Assay (EMSA)*

Probes for gel shift assays were labeled with ^{32}P in the presence of 〔γ-^{32}P〕 ATP and T4 polynucleotide kinase. Five molar excess of complementary oligonucleotide was added to the reaction mixture, and heated to 95 ˚C for 5 min, followed by cooling down to room temperature. The DNA sequence of double-stranded oligonucleotides for the probe was as follows;

LXRE , 5’ - CTGGCCCTGACCTTGTGACACTAGGCAGGG -3’

(only one strand is shown). The oligonucleotides used were as competitors (sense strand only) and as follows;

Rat CYP7A1; 5’ – gatcCTTTGGTCACTCAAGTTCAAGT – 3’.

The labeled probes (100,000cpm) was combined with nuclear extract in 25 mM Tris/HCl, pH7.4, 0.1 mM EDTA, 1 mM dithiothreitol, and 10 % glycerol. Binding reaction mixtures were incubated for 30 min on ice and resolved on a nondenaturing 4 % acrylamide gel (29:1 (w/w) acrylamide/ bisacrylamide) on 0.5× TBE (45 mM Tris, 45 mM boric acid, 1 mM EDTA). The dried gels were exposured to X-ray film at -70˚C with an intensifying screen.

5. *Semi quantitative RT-PCR*

Reverse transcription (RT)-PCRs were performed with the SuperScript First-Strand Synthesis System for RT-PCR (invitrogen) using 4μg of RNA. LGK and β-actin cDNAs were amplified for 30 cycles. RT-PCR products were separated by 1% agarose gel eletrophoresis. Primers used in this study were as followes;

GK s 5'-GGCTATGGATACTACAAGGT-3',

GK as 5'- TGACCAGCATCACTCTGAAG -3'

β-actin s 5' – TTGTAACCAACTGGGACGATATGG -3'

β-actin as 5' – CGACCAGAGGCATACAGGGACAAC -3'

6. *Quantitative Real time PCR*

Reverse transcription (RT)-PCRs were performed with the ImProme-Ⅱ ™ by using 4 μg of RNA. Quantitation of mRNA was performed with a quantitative, real-time PCR approach using Roche Lightcycler (Roche, Manngeim, Germany). A 20 $\mu\ell$ PCR reaction on the Roche Lightcycler contained 20 pmole of each primer, 2 $\mu\ell$ Lightcycler Master Mix, 2.4 $\mu\ell$ $MgCl_2$ and 1$\mu\ell$ template DNA. Cycling conditions included initial denaturation for 10 min at 95 ˚C, followed by 40 cycles of 10 sec at 95 ˚C , 10 sec at 58 ˚C and at 72 ˚C. The relative RNA quantitation was calculated using the comparative Ct method where ΔCt is $Ct_{\beta\text{-actin}} - Ct_{GK.}$ The Ct values were used to calculate 2^{Ct}.

7. *Animals*

To investigated whether activation of LXRs altered the expression of genes involved in glucose metabolism. Male *ob/ob* mice (8weeks old, approximately 50 *g* each) were housed and given water ad libitum. These mice intraperitoneal injections of either vehicle (control) or 50 ㎎ of TO-901317 /㎏ of body weight/day for 4 days. TO-901317 was dissolved in DMSO and dilute (3:1) with 0.9% saline prior to injection[16]. Glucose – tolerance tests were performed by i.p injection of glucose 2 g/㎏ body weight after 8 hr of fasting[17].

Ⅲ. Result

1. Effect of TO-901317 and 9 *cis*-retinoic acid on LGK expression in primary hepatocytes.

We investigated whether activation of LXRα altered the expression of genes involved in glucose metabolism. We treated LXRα ligand with primary hepatocytes isolated from male Sprague-Dawley rats (about 150 g) and measured the mRNA levels by northern blot. These data demonstrated that LGK transcript was increased by LXRα ligand, TO-901317 (Fig. 1).

2. Activation of the rat LGK promoter by LXRα/RXRα in Alexander cell.

In order to know LXRα can activate rat LGK promoter, we performed transient transfection assay using luciferase reporter construct under control of LGK promoter spanning from -993 to +127 (pRGKL-993). As shown in Fig. 2, the LGK promoter activity was significantly stimulated by expressions of LXRα and RXRα. Also LXRα ligand TO-901317, which induced the expression of the LGK gene in primary hepatocytes, can potentiate the LGK promoter activity. These observations led us to investigate whether LGK is a direct target of LXRα.

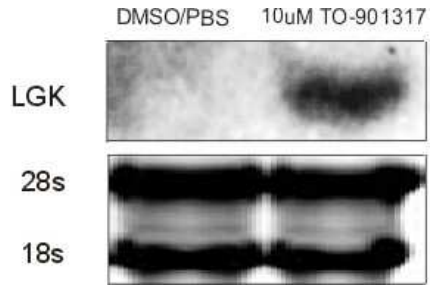


Figure 1. LXRα ligand induces transcription of the LGK gene in primary hepatocytes. Northern blot analysis of LGK mRNA in the rat primary hepatocytes. Cultured hepatocytes were treated for 24 hr in the presence or absence of TO-901317(10*u*M) and 9-*cis* retinoic acid (10*u*M). Total RNA isolated from primary hepatocytes were subjected to 0.9% formaldehyde agarose gel electrophoresis. RNA in the gel was transferred to nylon membrane and hybridized to ^{32}P labeled cDNAs for LGK . The 28s and 18s were used as an internal control.

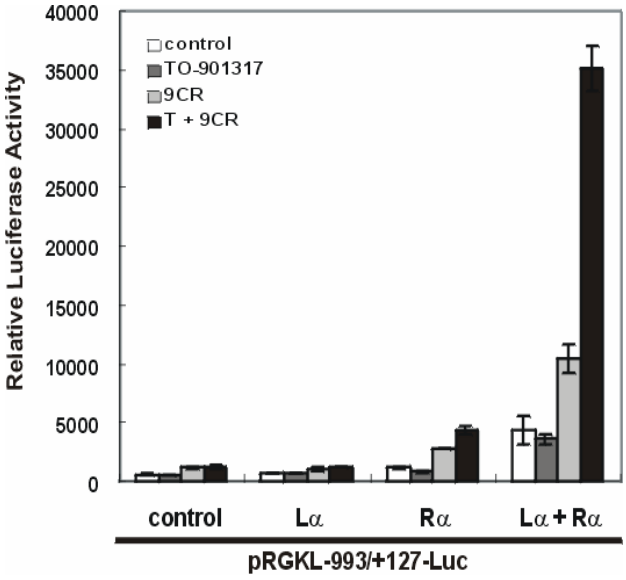


Figure 2. The LGK promoter is activated by overexpression of LXRα/RXRα. pRGKL-993, LGK promoter spanning -993 to +127, were transfected into Alexander cells with LXRα/RXRα. Each reporter constructs (0.5μg) was cotransfected with overexpression plasmid(0.1μg) of pCMX LXRα, pCMX RXRα or pCMX and reference construct (0.1μg) of pCMV β-gal. The cells were incubated for 24 hr after transfection and treated with LXRα ligand TO-901317(1*u*M), RXRα ligand 9 *cis*-retinoic acid (1*u*M) for 24 hr. After 24hr, the luciferase activities were assayed and were normalized with with β-galactosidase activities.

3. Localization of LXRE in the LGK promoter

To identify potential LXRα response element in the LGK promoter, we prepared 5' serial deletion constructs and performed transient transfection assay. We can estimate that LXRE exists between -120 and -25 through the deletion study (upper panel). However, if we present this data through fold activation, We can observe serial reduction of activation fold between -238/-25 (lower panel). Deletion between -238 and -120 resulted in 38 % reduction in activity. Deletion between -120 and -76 showed 50 % reduction and deletion between -76 and -25 showed 57 % reduction in activity (Fig. 3). These three sites are thought to be related to LXRα dependent activation of LGK promoter. SREBP-1c and PPARγ induce GK gene transcription through binding to SREs and PPRE in the LGK promoter each other[14, 15]. LXRα is known to activate the transcription of SREBP-1c, PPARγ. SRE and PPRE located in -205/-176 and -116/-104 region, respectively. Thus we assumed LXRα can activate LGK promoter indirectly through SRE and PPRE and -76/-25 region is also responsible for LXRα dependent activation of LGK promoter. As a result, we assumed that SREBP-1c , PPARγ and LXRα might be related to the regulation LGK promoter.

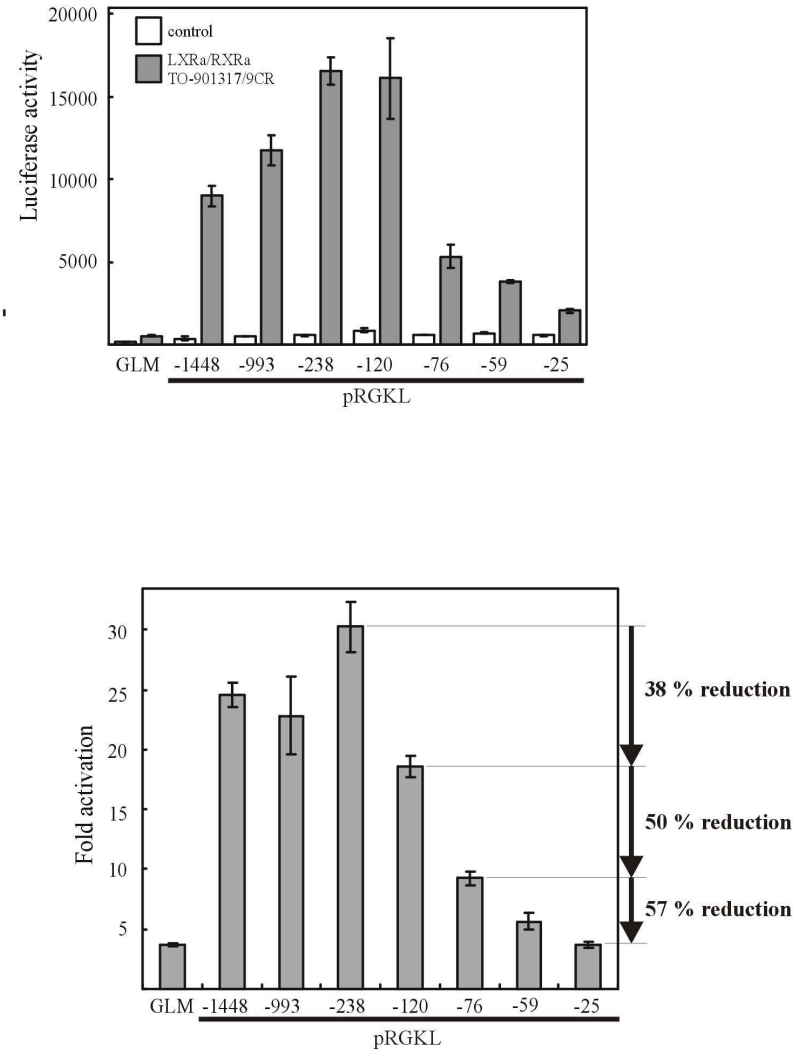


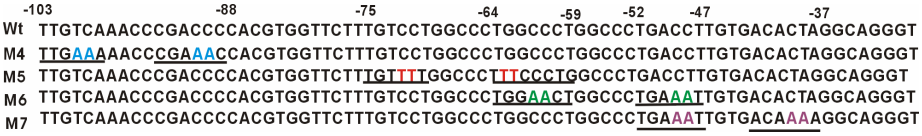
Figure 3. Rat LGK promoter was activated by LXRα/RXRα. 5' serial deletion constructs of the LGK promoter luciferase reporter were transfected into Alexander cell with (gray bars) or without (white bars) expression of LXRα/RXRα with ligand.

Normalized luciferase activities were expressed in upper panel and fold increase relative to the basal activity without LXRα/RXRα expression in lower panel. Each reporter constructs (0.5 ug) was cotransfected with overexpression plasmid(0.1ug) of pCMX LXRα, pCMX RXRα or pCMX and reference construct (0.1ug) of pCMV β-gal. The cells were incubated for 24 hr after transfection and treated with LXRα ligand TO-901317 (1*u*M), RXRα ligand 9 *cis*-retinoic acid (1*u*M) for 24 hr.

4. Effect of putative LXRE mutation on the LGK promoter activity

Sequence analysis could not predict possible DR4+ sequence in -103/-29 region. We performed computer based sequence analysis to search for the LXRE in -103/-29 region of LGK promoter. There are 5 'AGGTCA' similar sequence in -103/-29 region. We introduced mutation into the those 'AGGTCA' sequence (Fig. 4A), and tested LXRα response. As shown in fig 4B, m6 and m7 mutation resulted in loss of LXRα responsiveness. We thought the region overlapping m6 and m7 mutation is possible LXRE. To further confirm the putative LXRE, we truncated the -52/-37 region. This truncated mutant promoter could not response to LXRα. In addition, we observed that PPRE mutation(m2) could decrease LXRα dependent activation of LGK promoter. We made dual truncation mutant to confirm the effect of PPRE. Truncation of PPRE and putative LXRE resulted in the promoter activity almost down to the basal level.

(A)



(B)

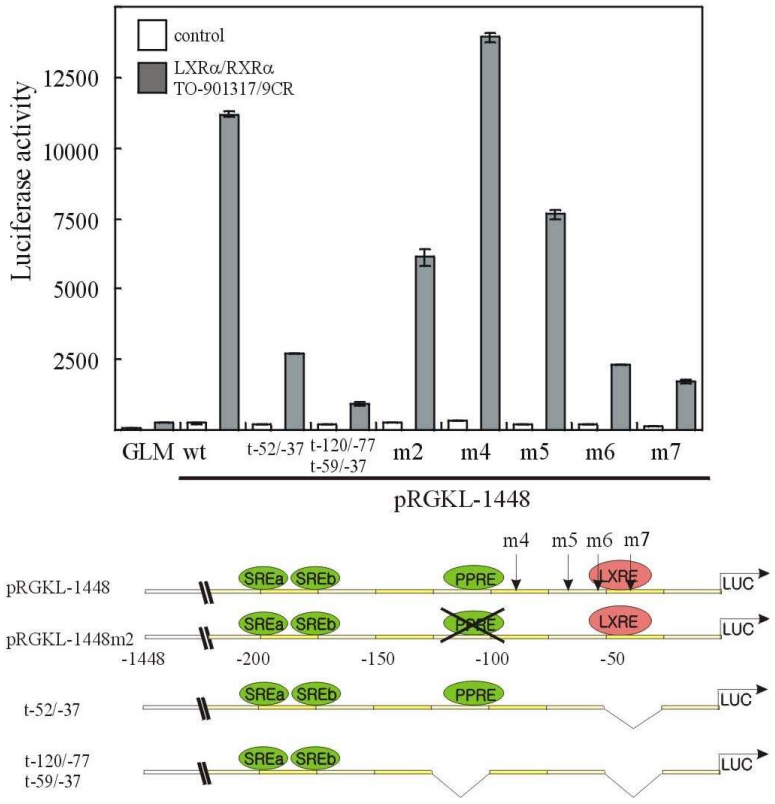


Figure 4. The effects of mutations and truncations on LGK promoter. A) sequence alignment of putative LXRE and DNA sequence mutant versions are shown in the rat LGK promoter. B) Wild type, its putative LXRE and truncation were transfected into

Alexander cells with (gray bars) or without (white bars) LXRα/RXRα overexpressing constructs. The cells were incubated for 24hr after transfection and treated with LXRα ligand TO-901317(1*u*M), RXRα ligand 9 *cis*-retinoic acid(1*u*M) for 24hr. Luciferase activity were normalized by β-galactosidase activities.

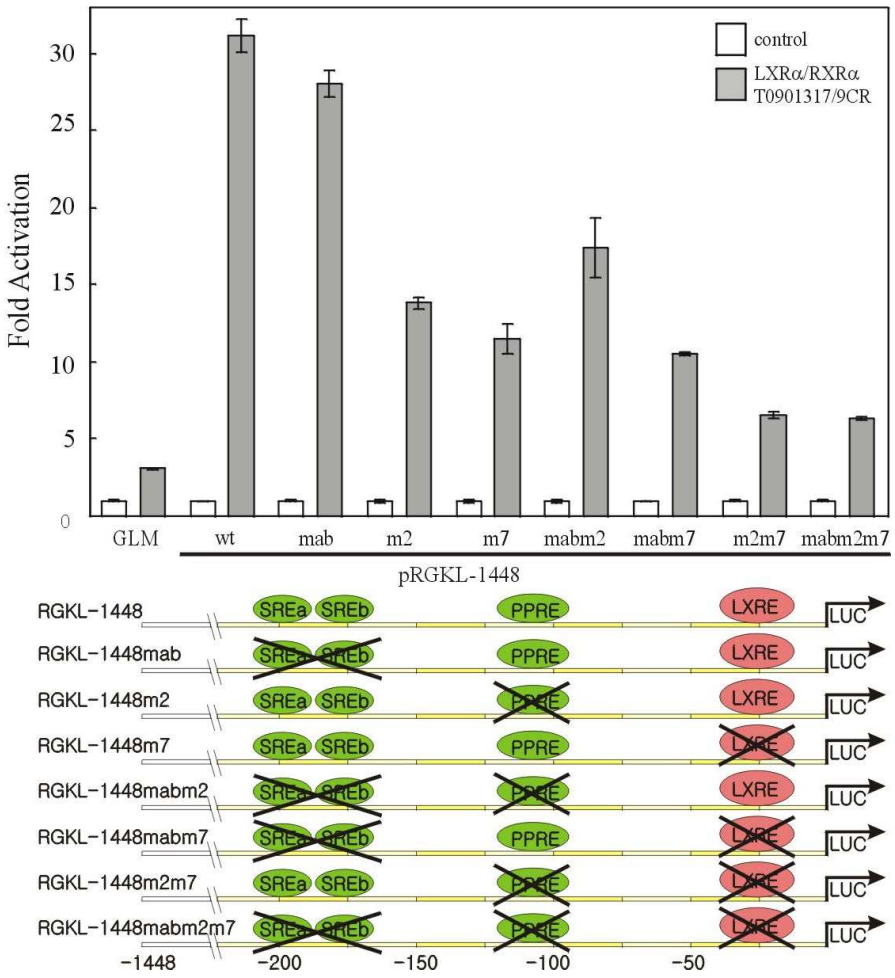


Figure 5. LXRα induce the expression of the LGK gene *via* direct activation and via SREBP-1c and PPARγ. Wild or mutant type rat LGK promoter luciferase reporter constructs were transfected into Alexander cells with (gray bars) or without(white bars) overexpression of LXRα/RXRα. Mutant constructs RGKL-1448mab (SRE matant), RGKL-1448m2 (PPRE mutant), RGKL-1448m7 (LXRE

mutant), RGKL-1448mabm2, RGKL-1448mabm7, RGKL-1448m2m7, and RGKL-1449mabm2m7 were produced by site directed mutagenesis technique. The cells were incubated for 24hr after transfection and treated with LXRα ligand TO-901317(1*u*M), RXRα ligand 9 *cis*-retinoic acid(1*u*M) for 24 hr. The luciferase activities were shown as the values normalized by β-galatosidase activities.

5. Effects of mutations on SRE, PPRE, and LXRE on LXRα/RXRα mediated activation in rat LGK promoter.

It is reported that LGK is a direct target gene of SREBP-1c and PPARγ, which induce the expression of the LGK gene via direct activation of the LGK promoter. In addition, LXRα activation led to the induction of both SREBP-1c and PPARγ. Furthermore, activated SREBP-1c produce unsaturated fatty acid which is a ligand of PPARγ. Assuming that LXRα-dependent activation of SREBP-1c and PPARγ play a critical role in the LGK promoter activity, we performed mutation studies to find out their correlation with LGK promoter.

To understand how activated LXRα enhance LGK promoter activity with SREBP-1c and PPARγ, we prepared complex reporter constructs.

SRE mutation (mab), did not result in the significant reduction in LXRα driven promoter activity. PPRE mutation (m2) caused about 30 % reduction in activity. LXRE mutation (m7) showed more that 60 % reduction in the promoter activity. Dual mutation of SRE and PPRE (mabm2) showed similar result with PPRE mutant (m2)

and SRE, LXRE double mutant (mabm7) showed similar results with LXRE mutant (m7). Double mutation of PPRE and LXRE (m2m7) and triple mutation of SRE, PPRE and LXRE (mabm2m7) showed almost complete disappearance of LXRα dependent activation of LGK promoter. These results suggested that LXRα can activate LGK promoter not only directly through direct binding to LXRE but also indirectly through activating SREBP-1c and/or PPARγ. Our result also suggested that LXRE and PPRE is primarily responsible for LXRα dependent activation.

6. Direct binding of LXRα/RXRα to the rat LGK promoter.

To see whether LXRα binds directly to the LGK promoter, we performed EMSA experiment using -59/-30 fragment of LGK promoter. For this study, we overexpressed LXRα or RXRα in the NIH-3T3 cells and prepared nuclear extract from the cells. As shown in the Fig. 6, nuclear extract from LXRα overexpressed NIH-3T3 cell showed protein-DNA binding. However, RXRα overexpressed nuclear extract did not showed protein-DNA binding. Because it is known that LXRα was not expressed in the NIH-3T3[18] and thus, LXRα overexpression might be enough for LXRα/RXRα heterodimer and protein-DNA interaction. When we mixed LXRα overexpressed nuclear extract and RXRα overexpressed nuclear extract, we can observe strong protein-DNA interaction and this interaction could be compete out by unlabeled LXRE containing oligonucleotide (CYP7A1). This result indicated the binding of LXRα/RXRα heterodimer to LXRE of LGK promoter.

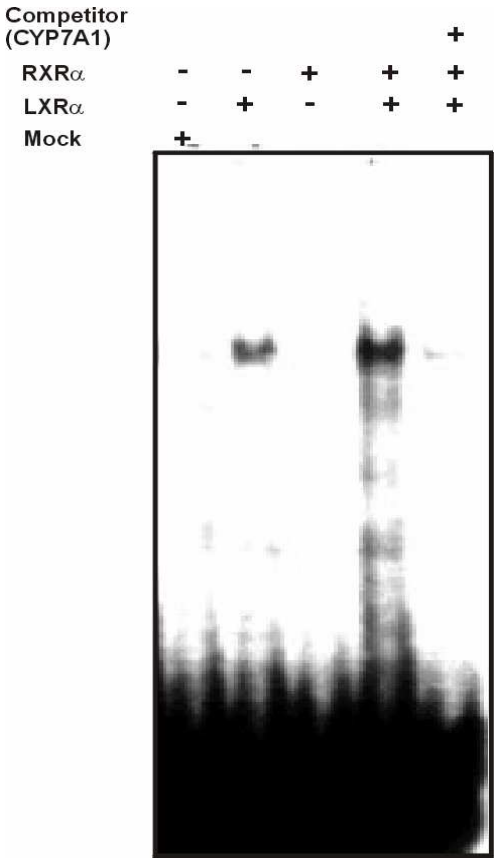


Figure 6. LXRα/RXRα bind to the proximal region of the LGK promoter. Electrophoretic mobility shift assays were performed by using NIH-3T3 nuclear extract and ^{32}P-labeled LGK LXRE oligonucleotide (-59/-30). unlabeled 100 molar excess CYP7A1 LXRE was added as competitor. Probes (100,000cpm of [α -^{32}P] ATP-labeled rat LGK promoter) were incubated in the reaction mixture with 6 μg nuclear extract for 30 min on ice.

7. Effect of TO-901317 on glucose tolerance in *ob/ob* mice.

Finally, we have investigated the effect of LXRα ligand on the LGK expression and blood glucose level. Intraperitoneal treatment of TO-901317 to *ob/ob* mouse showed the improvement in the glucose tolerance test (Fig. 7) and increased GK expression in liver. These results demonstrated that activation of the LXR signaling pathway modulates blood glucose level via LGK gene activation.

8. Direct regulation of LGK by LXRα ligand independent on insulin

To know the effect of LXRα on endogenous LGK expression, we treated rat primary isolated hepatocytes with LXRα ligand, TO-901317 for 24 hours in the presence of absence of serum. LXRα ligand can increase LGK transcription and serum can further potentiate the LGK expression.

There is a report that LXRα can induce SREBP-1c gene expression[2, 19], but can not induce mature nuclear form. Precursor form that are increased by LXRα need insulin to become mature form[3, 11]. In this group, it explains the induction of LGK by LXRα is SREBP-1c dependent. Using these datas, we performed real time PCR to see the levels of LGK, SREBP-1c, LXRα, and PPARγ when insulin is depleted which is a state that precursor form of SREBP-1c is induced by LXRα but no increase on mature form. Even though mature form of SREBP-1c is not increased, you can an increase in LGK due to LXRα ligand. We could confirm that LGK is controlled directly through LXRα and independent from SREBP-1c. The mRNA level of SREBP-1c was increased

through LXRα. We could not see the difference of PPARγ or LXRα due to LXRα ligand with or without serum. From these results, we concluded that LXRα ligand can upregulate LGK expression directly by activating of LXRα and indirectly by activation of SREBP-1c and PPARγ.

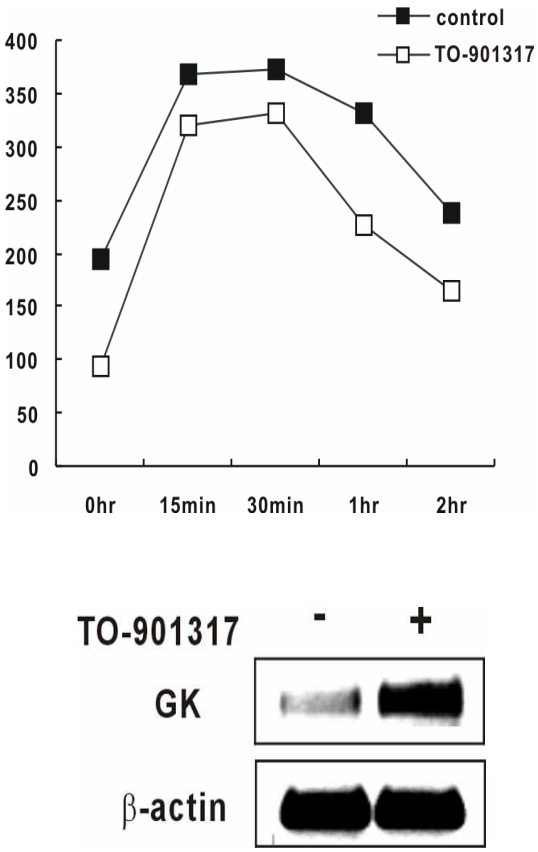
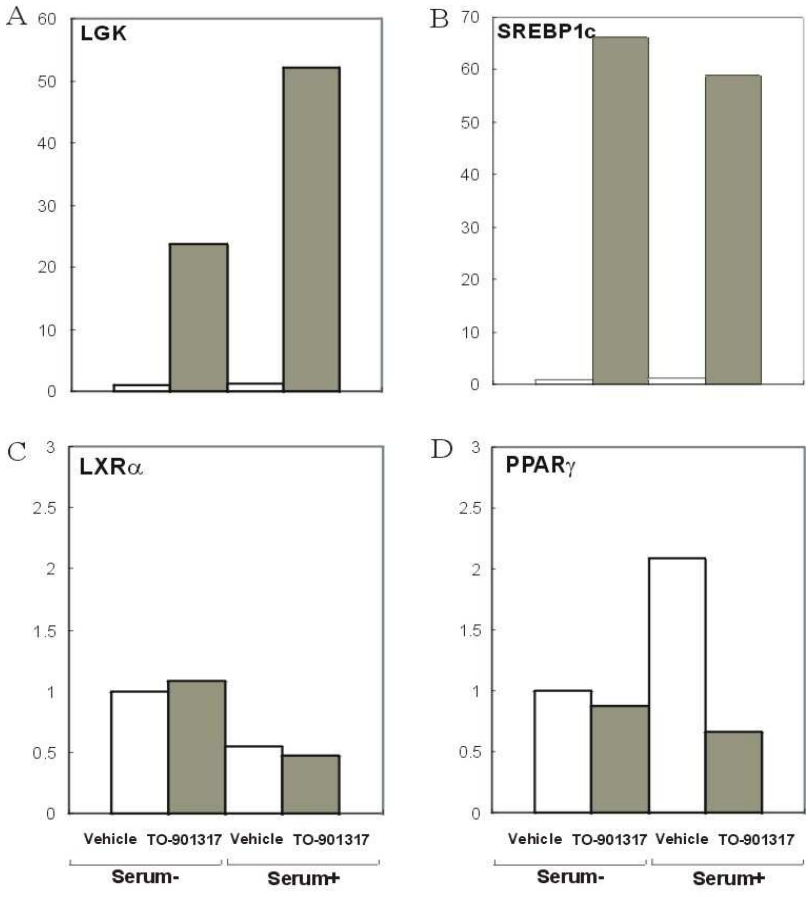


Figure 7. The blood glucose level were decreased by activation of LGK mRNA. LXRα-mediated *ob/ob* mice were treated with TO-901317(50 mg/kg) or vehicle for 4 days. Glucose – tolerance tests were performed by i.p injection of glucose 2 g/kg body weight after 8 hr of fasting. Liver tissue collected, and total RNA was isolated for RT-PCR analysis to examine the mRNA expression of LGK.


A
LGK
B
SREBP1c
C
LXRα
D
PPARγ
Vehicle
TO-901317
Vehicle
TO-901317
Serum-
Serum+
Relative mRNA levels

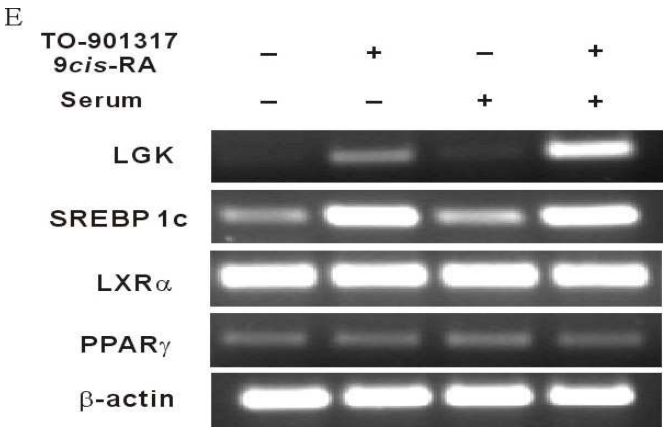

E
TO-901317
9cis-RA
Serum
LGK
SREBP 1c
LXRα
PPARγ
β-actin

Figure 8. Relative expression of LGK, SREBP-1c, LXRα and PPARγ mRNA in primary hepatocytes treated with serum or the LXR agonist TO-901317. Hepatocytes were maintained overnight in basal medium before treatment with serum, TO-901317 (10 *u*M ; gray bars), or DMSO/PBS(vehicle control ; white bars) for 24 hr. The relative RNA quantitation was calculated using the comparative Ct method where ΔCt is $Ct_{\beta\text{-actin}} - Ct_{GK.}$ The Ct values were used to calculate 2^{Ct}.

Ⅳ. Discussion

The activation of LGK led to an increase in the intracellular concentration of glucose-6-phosphate, which was also related to an induction of glycogenesis and glycolysis pathway[1]. In the liver, the major function of glycolysis may not provide pyruvate to be oxidized in the citric acid cycle but to allow the transformation of carbohydrate into fat. In diabetic rats, the activity of glucokinase and glucose phosphorylating capacity of the liver are low. Concomitantly, the levels of hepatic glycolysis was decreased.

Bronwyn D. Hegarty *et al.* has reported that full induction of the mature and transcriptionally active form of SREBP-1c protein requires insulin[11]. Although activation of LXRα lead to the induction of SREBP-1c gene expression and precursor protein, it has a very poor effect in inducing the mature nuclear form. These authors suggested that LGK expression by LXRα ligand was increases through SREBP-1c activated by insulin, because SREBP-1c induced LGK gene transcription through binding to the SREs in the LGK promoter. But they seemed to pass over the LXRα/RXRα to bind to the element and direct activation of the LGK promoter. Actually, we observed that the LGK mRNA continued to increase by LXRα ligand, despite a lack of serum (including insulin). This result suggest that LGK is a direct target gene of LXRα.

We thought that LXR-activated PPARγ expression was affected to LGK promoter. Because SEO et al. reported that the expression level of PPARγ mRNA was enhanced with the LXR agonist in liver[16]. By contrast, in our studies the expression of PPARγ was not activated by LXR agonist in primary hepatocyte. So far, we have no explanation. But the LXRα ligand effect decreases as the PPRE is mutated. If PPRE is mutated LXRα cannat be activated through PPARγ. The remaining effect of the LGK promoter activity might be caused by the direct effect of LXRα to the promoter.

Others have reported that LXRα expression in the liver is induced by insulin[20]. By contrast, Bryan A, Laffitte has reported that there was no difference between fasted and fed animals on the expression of LXRα, even though SREBP-1c expression was strongly regulated[17]. Another group demonstrated that TO-901317, an LXRα agonist, did not appear to work through the classic insulin signaling cascade. In addition Guoqing Cao et al, found that LXR activation does not alter Akt phosphorylation[13]. Actually, our studies revealed that the levels of LXRα mRNA did not change. There seemed to be a change in LXR activity, but not in the quantity. It is suggested that LXRα effects on glucose metabolism would need to be separated from SREBP-1c-dependent effects on lipogenesis before LXRα agonist would be useful as antidiabetic agent. Because LXR ligand dramatically raises plasma triglyceride levels and leads to profound hepatic steatosis[21]. But we thought that the LXRα-mediated SREBP1c effect was determined by insulin.

We hypothesized how activated LXRα can enhance LGK promoter activity with

SREBP1c and PPARγ. First, activated LXRα directly binds with LXRE in LGK promoter which is well conserved. Second, LXRα activation substantially increased the expression of SREBP-1c mRNA, which is affected to LGK promoter. Third, It is reported that PPARγ is a novel target of LXRα. However, the expression level of PPARγ mRNA was not enhanced by LXRα ligand in primary hepatocytes. We think that LXRα-stimulated SREBP1c produced the PPARγ ligand and then activated PPARγ induced LGK gene expression.

In this study, not only did LXRα bind directly to the LXRE motif in the LGK promoter, but it also stimulated LGK promoter activity via direct activation and via SREBP-1c and PPARγ activated by LXRα ligand. TO-901317, presumably functioning as an LXRα agonist, effectively lowers glucose in *ob/ob* mice.

Ⅴ. Conclusion

1. Activation of LXRα with TO-901317 lead to the induction of hepatic glucokinase expression.

2. LXRE (Liver X receptor response elements) could be located between -59 and -30

3. LXRα induce the expression of the LGK gene via direct activation and via SREBP-1c and PPARγ stimulated by LXRα ligand.

4. LXRα signaling pathway modulates blood glucose level *via* LGK gene activation.

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Abstract(in korea)

LXRα에 의한 liver type glucokinase 전사조절

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김태현

LGK(Liver type glucokinase)는 liver에서의 glucose이용과 glycolysis와 glycogenesis과정에서 중요한 역할을 담당하므로 liver에서 glucose sensing에 있어서 LGK의 조절은 매우 중요하다. Nuclear receptor의 종류인 LXR (Liver X Receptor)은 α form과 β form이 존재하는데, lipid metabolism과 cholesterol metabolism에 관여된 gene expression을 조절하는 것으로 알려져 있다. 이번 연구에서 LXRα 가 LGK의 발현을 증가시킴으로 glucose homeostasis 에 중요한 역할을 담당한다는 것을 밝히고자 한다. LXRα를 활성화 시키는 synthetic ligand인 TO-901317은 hepatic glucokinase의 발현을 증가시키고, LXRα와 heterodimer를 이루는 RXRα와 LXRα는 LGK promoter에 직접 binding해서, LGK promoter activity를 높인다. LGK promoter에서 -52/-37부위가 LXRα가 binding할 수 있는 LXRE를 찾아 직접적으로 영향을 주는 것을 밝혔다. LXRα ligand에 의해 유도된 SREBP-1c와 PPARγ를 통해서도 LGK promoter가 활성화 됨을 확

인하였다. 또한 LXRα ligand인 TO-901317을 *ob/ob* mice에 처리했을 때 혈중 glucose 농도가 낮아짐을 알 수 있었다. 이러한 결과로 LXRα는 direct 하게, indirect하게 liver에서 LGK 발현을 증가시킴을 알 수 있었고, 이는 glucose homeostasis를 유지하는데 영향을 줌을 알 수 있었다.

핵심 되는 말 : Liver type glucokinase (LGK), Liver X receptor (LXR), glucose homeostasis, 전사조절