College of Pharmacy and Institute of Pharmaceutical Science and Technology[1], Hanyang University, Ansan, Republic of Korea; College of Pharmacy[2], Seoul National University, Seoul, Republic of Korea

Nectandrin B, a lignan isolated from nutmeg, inhibits liver X receptor-α-induced hepatic lipogenesis through AMP-activated protein kinase activation

Du Gon Choi[1,*], Eun Kyung Kim[1,*], Jin Won Yang[2], Jae Sook Song[1], Young-Mi Kim[1]

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Young-Mi Kim, Department of Pharmacy, College of Pharmacy and Institute of Pharmaceutical Science and Technology, Hanyang University, 55 Hanyangdaehak-ro, Sangnok-gu, Ansan, Gyeonggi-do, 426-791, Republic of Korea ymikim12@hanyang.ac.kr

[*]*These authors contributed equally to this work.*

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Nonalcoholic fatty liver disease is recognized as the most commonly occurring chronic liver disease. Liver X receptor α (LXRα) and sterol regulatory element-binding protein (SREBP)-1c play a central role in *de novo* fatty acid synthesis. This study investigated pharmacological effects of nectandrin B, a lignan isolated from nutmeg extract, on hepatic lipogenesis stimulated by LXRα-SREBP-1c-mediated pathway and the possible molecular basis. The reporter gene assay revealed that nectandrin B completely represses LXRα activity enhanced by a synthetic LXRα ligand (T0901317) in HepG2 cells. The inhibitory effect was further supported by the suppression of mRNA expression of LXRα target genes, SREBP-1c and LXRα itself. Nectandrin B also inhibited the increase in SREBP-1c expression promoted by insulin plus high glucose, major contributors to hepatic lipid accumulation. LXRα-SREBP-1c-mediated induction of acetyl-CoA carboxylase 1 and fatty acid synthase, major genes for *de novo* lipogenesis, was suppressed by nectandrin B. Moreover, Oil Red O staining showed that nectandrin B notably attenuates LXRα-induced lipid accumulation. AMP-activated protein kinase (AMPK) inhibits the activities of LXRα and SREBP-1c. Nectandrin B strongly activated AMPK signaling in HepG2 cells. Taken together, the suppressive effects of nectandrin B on lipogenic gene expression and lipid accumulation in hepatocytes may be due to its inhibitory effect on the LXRα-SREBP-1c pathway presumably *via* AMPK activation. These results suggest the potential of nectandrin B as a therapeutic candidate for fatty liver disease.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is recognized as the most commonly occurring chronic liver disease (Asrih and Jornayvaz 2015). Dysregulation of hepatic fatty acid metabolism can cause excess lipid accumulation in hepatocytes, a major characteristic of NAFLD (Fabbrini et al. 2010). A previous study in patients with NAFLD demonstrated that about 26% of fatty acids in triglycerides produced in liver originate from *de novo* hepatic fatty acid synthesis, suggesting the significant contribution of *de novo* lipogenesis pathway to hepatic triglyceride accumulation (Donnelly et al. 2005). Furthermore, hepatic expression of major lipogenic genes, such as acetyl-CoA carboxylase (ACC) 1 and fatty acid synthase (FAS) is enhanced in parallel with the increase in their key transcription factors, liver X receptor α (LXRα) and sterol regulatory element binding protein (SREBP)-1c in NAFLD patients (Higuchi et al. 2008).

LXRα is a nuclear receptor activated by endogenous oxysterols and serves as a crucial regulator of cholesterol, fatty acid, and glucose metabolism (Calkin and Tontonoz 2012; Jakobsson et al. 2012). LXRα plays an essential role in the regulation of hepatic *de novo* fatty acid synthesis through the transactivation of SREBP-1c, a master transcription factor for the major genes involved in *de novo* lipogenesis (Schultz et al. 2000; Repa et al. 2000; Shimomura et al. 1998). Moreover, LXRα increases FAS gene expression by directly binding to its promoter region (Joseph et al. 2002). Interestingly, LXRα is required for insulin-stimulated SREBP-1c gene transcription and fatty acid synthesis (Chen et al. 2004).

AMP-activated protein kinase (AMPK) as a critical regulator of glucose and lipid metabolism, reduces the synthesis of fatty acid and cholesterol in the liver, but promotes fatty acid oxidation in skeletal muscle (Lage et al. 2008). ACC1, a rate-limiting enzyme in hepatic fatty acid synthesis pathway is directly inactivated *via* AMPK-mediated phosphorylation (Lage et al. 2008). Furthermore, AMPK suppresses the expression of lipogenic genes by regulating the transcriptional activities of LXRα and SREBP-1c through direct phosphorylation (Hwahng et al. 2009; Li et al. 2011).

Nutmeg, the dried semen of *Myristica fragrans* Houtt. (Myristicaceae) is a popularly used spice and has also been used as a traditional medicine for the treatment of diarrhea (Nguyen et al. 2010; Van Gils and Cox 1994; Grover et al. 2002). Several pharmacological activities of nutmeg extracts, such as

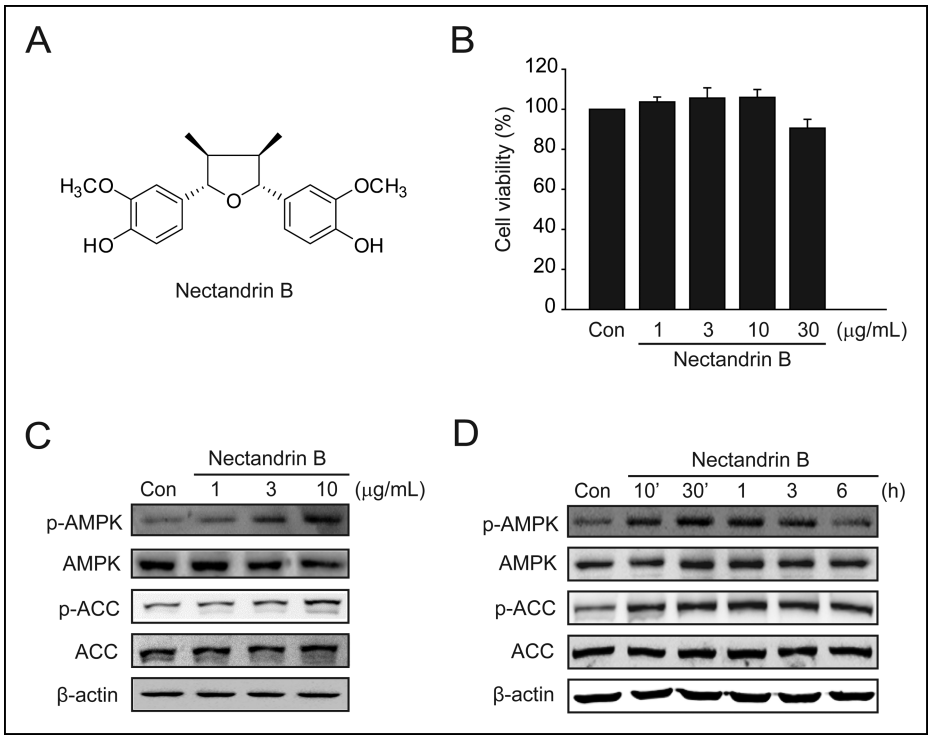

Fig. 1: Effect of nectandrin B on cell viability and AMPK signaling in HepG2 cells. (A) Chemical structure of nectandrin B. (B) Cells were incubated with the indicated concentrations of nectandrin B for 24 h, and then the cell viability was assessed by MTT assay. Data represent the mean ± S.E. (n = 4). (C-D) Cells were exposed to the indicated concentrations of nectandrin B for 1 h or treated with nectandrin B (10 μg/mL) for the indicated times. Cell lysates were obtained and each protein level was determined by Western blot analysis.

anti-inflammatory, anti-hyperlipidemic, and anti-atherosclerotic effects have been reported (Olajide et al. 1999; Ram et al. 1996; Sharma et al. 1995). In a previous study, a total extract of nutmeg was shown to activate AMPK and seven tetrahydrofuran-type lignans were isolated from this extract as active constituents (Nguyen et al. 2010). Among those lignans, nectandrin B exhibits a strong activating effect on AMPK signaling in differentiated C2C12 cells (Nguyen et al. 2010).

In the present study, we investigated the pharmacological effects of nectandrin B on hepatic lipogenesis. We determined if nectandrin B activates AMPK signaling pathway in hepatocytes. We also evaluated its ability to inhibit the transcriptional activity of LXRα and down-regulate LXRα-mediated lipogenic gene expression. Furthermore, we report that nectandrin B attenuates lipid accumulation stimulated by an LXRα ligand in hepatocytes.

2. Investigations and results

2.1. Effect of nectandrin B on HepG2 cell viability

An MTT colorimetric assay was used to examine the cytotoxicity of nectandrin B in HepG2 cells. Cell viability tended to slightly decrease at a concentration of 30 μg/mL (Fig. 1B). Thus, 1-10 μg/mL of nectandrin B was treated for subsequent experiments.

2.2. Activation of AMPK signaling by nectandrin B in HepG2 cells

Many studies have demonstrated the critical roles of AMPK in the regulation of lipid metabolism, particularly the inhibitory effects on LXRα and SREBP-1c activities (Lage et al. 2008; Hwahng et al. 2009; Li et al. 2011). AMPK was notably activated in response to the treatment of nectandrin B in HepG2 cells as evidenced by the increase in the phosphorylation of AMPK at Thr172 (Fig. 1C and 1D). This result was also confirmed by the increased phosphorylation of ACC, a downstream substrate of AMPK. Nectandrin B enhanced the phosphorylation of AMPK and ACC in a concentration-dependent manner (Fig. 1C). The concentration of 10 μg/mL of nectandrin B capable of strongly activating AMPK was applied to the subsequent experiments unless specified otherwise. The levels of phosphorylated forms of AMPK and ACC began to increase from 10 min after treatment of 10 μg/mL of nectandrin B and the effects were maintained up to at least 3 h. These results demonstrate that nectandrin B can activate AMPK in hepatocytes, implying the possibility of its inhibitory action on hepatic lipogenesis.

2.3. Preventive effect of nectandrin B on hepatic SREBP-1c induction

SREBP-1c serves as a common transcription factor for the induction of major lipogenic enzymes, thus leading to hepatic steatosis (Shimomura et al. 1998; Moon et al. 2012). LXRα plays a central role in the transcriptional regulation of SREBP-1c (Repa et al. 2000). We therefore used T0901317 (a synthetic LXRα ligand) to induce SREBP-1c expression and hepatic lipogenesis in the present study.

In order to investigate the ability of nectandrin B to prevent hepatic lipogenesis, we first determined the effect of nectandrin B on the SREBP-1c induction stimulated by T0901317. Nectandrin B abrogated T0901317-induced SREBP-1c up-regulation at the concentration of 10 μg/mL (Fig. 2A). The inhibitory effect of nectandrin B on the LXRα-dependent SREBP-1c induction was confirmed by the study using GW3965, a more specific LXRα ligand (Fig. 2B). Moreover, we observed the ability of nectandrin B to attenuate the notable increase in SREBP-1c protein expression after exposure to insulin plus high glucose, major contributors to the pathogenesis of hepatic lipid accumulation in metabolic syndromes (Fig. 2B). Our results suggest that nectan-

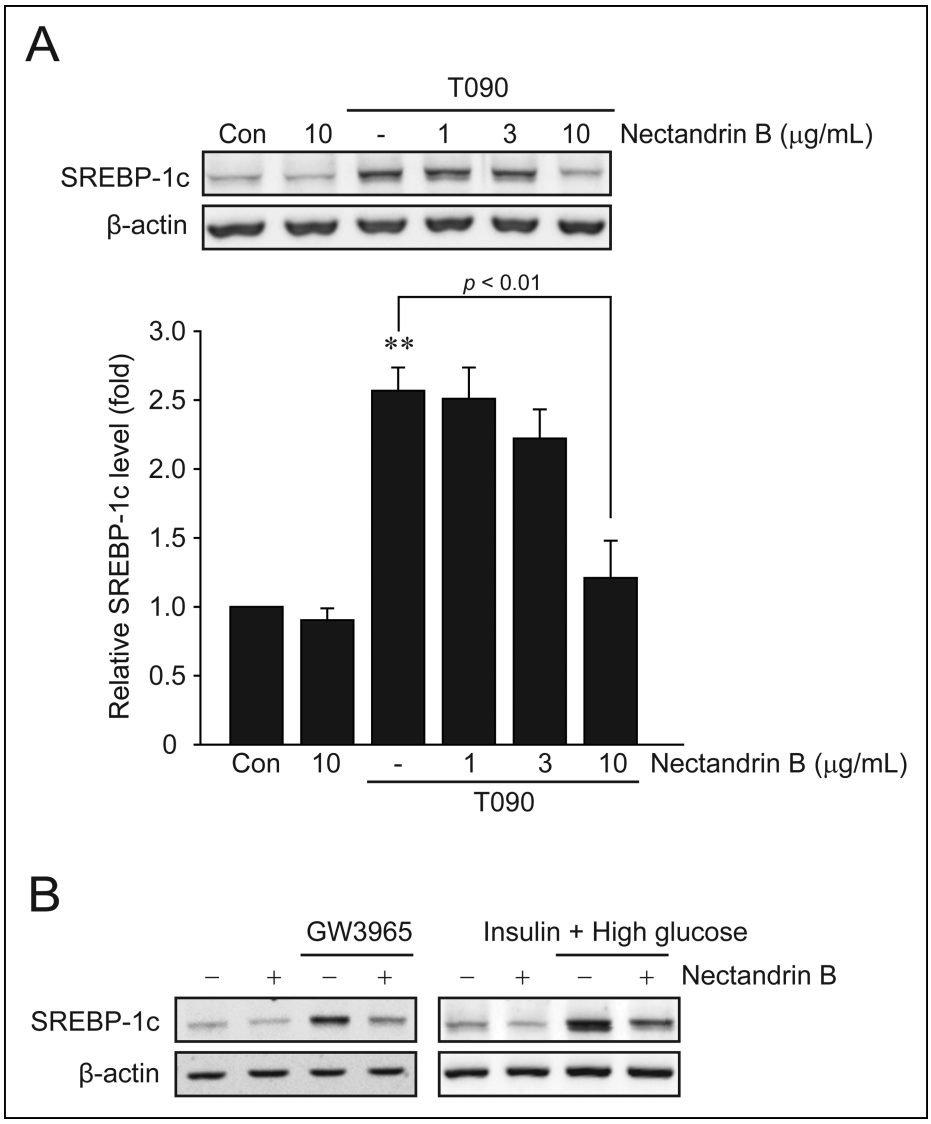

Fig. 2: Effect of nectandrin B on SREBP-1c induction by LXRα agonists or insulin plus high glucose in HepG2 cells. (A) Cells were pretreated with vehicle or nectandrin B (1-10 μg/mL) for 1 h and subsequently exposed to T0901317 (T090, 10 μM) for 12 h. The cell lysates were then immunoblotted and band intensity was determined by scanning densitometry. The SREBP-1c levels were normalized to those of β-actin. Data represent the mean ± S.E. (n = 5). $^{**}P < 0.01$ (compared with the vehicle-treated control) (B) Cells were pretreated with nectandrin B (10 μg/mL) for 1 h and then incubated with GW3965 (10 μM) for 12 h, or insulin (100 nM) plus high glucose (25 mM) for 24 h. The SREBP-1c protein levels were measured in the cell lysates.

drin B can block the LXRα-dependent transcriptional induction of SREBP-1c.

2.4. Inhibitory effect of nectandrin B on LXRα transcriptional activity and LXRα-mediated lipogenic gene induction

We next measured the LXRE luciferase activities to verify the inhibitory effect of nectandrin B on LXRα transactivation. The pTK-CYP7A1-LXRE(x3)-LUC reporter gene construct including three copies of an LXRE sequence in rat *Cyp7a1* gene was used for this study. The reporter gene assay revealed that nectandrin B significantly attenuates the ability of T0901317 to enhance the CYP7A1-LXRE-luciferase activity (Fig. 3A).
Multiple LXREs are found in human LXRα gene promoter and LXRα can autoregulate its own promoter (Laffitte et al. 2001). Accordingly, the mRNA expression of LXRα was increased by 12 h exposure of HepG2 cells to T0901317, and pretreatment with nectandrin B completely abrogated this increase (Fig. 3B left). Similarly, nectandrin B repressed the T0901317-induced mRNA expression of SREBP-1c, another LXRα target gene (Fig. 3B right). Both FAS and ACC1, main enzymes for *de novo* fatty acid synthesis, are regulated by LXRα and SREBP-1c (Joseph et al. 2002; Lopez et al. 1996). As expected, nectandrin B significantly reduced the LXRα-SREBP-1c-mediated induction of FAS and ACC1 (Fig. 3C).
Taken together, these results demonstrate that nectandrin B inhibits the LXRα activity and subsequently suppresses the increase in hepatic lipogenic gene expression, implying its possible beneficial effect on hepatic lipid accumulation.

2.5. Attenuation of LXRα-induced lipid accumulation by nectandrin B in primary mouse hepatocytes

We further determined whether nectandrin B exerts antilipogenic effects in primary hepatocytes isolated from mice. In consistence with the results obtained in HepG2 cells, the inhibitory effect of nectandrin B on the T0901317-induced SREBP-1c protein expression was confirmed in primary cultured mouse hepatocytes (Fig. 4A). Next, we examined the effect of nectandrin B on the T0901317-stimulated lipid accumulation. The primary cultured mouse hepatocytes were stained

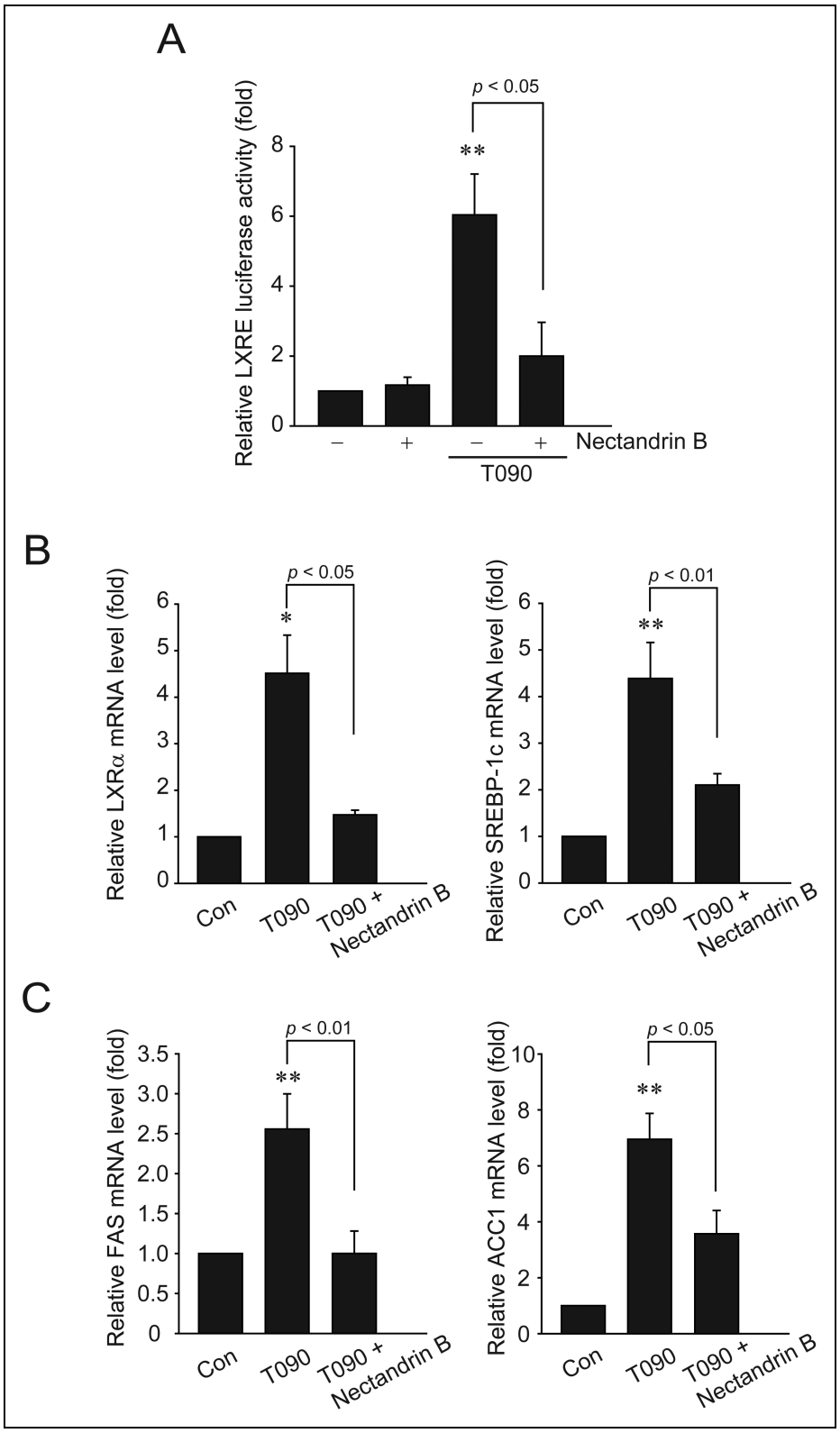

Fig. 3: Effect of nectandrin B on LXRα transcriptional activity and LXRα-mediated lipogenic gene induction in HepG2 cells. (A) Cells were transfected with an LXRE–luciferase construct and then exposed to T0901317 (T090, 10 μM) for 12 h after 1 h pretreatment with vehicle or nectandrin B (10 μg/mL). The relative LXRE luciferase activity was measured in the cell lysates. Data represent the mean ± S.E. (n = 4). $^{**}P < 0.01$ (compared with the vehicle-treated control) (B-C) Cells were treated with vehicle or 10 μg/mL nectandrin B for 1 h and subsequently exposed to T0901317 (T090, 10 μM) for 12 h. Each mRNA level was analyzed by using real-time PCR assay and normalized to that of GAPDH. Data represent the mean ± S.E. (n = 3-6). $^{*}P < 0.05$, $^{**}P < 0.01$ (compared with the vehicle-treated control).

with Oil Red O to visualize intracellular lipid droplets. Oil Red O staining revealed that LXRα-induced accumulation of lipid droplets was notably attenuated by the pretreatment of nectandrin B (Fig. 4B). These results indicate that nectandrin B has anti-steatotic effects through the inhibition of LXRα-mediated lipogenic gene induction in hepatocytes.

3. Discussion

The liver is a primary organ in regulating carbohydrate and lipid metabolism to maintain whole-body energy homeostasis (Fabbrini et al. 2010). Nonalcoholic simple fatty liver can progress to nonalcoholic steatohepatitis (NASH), a more severe condi-

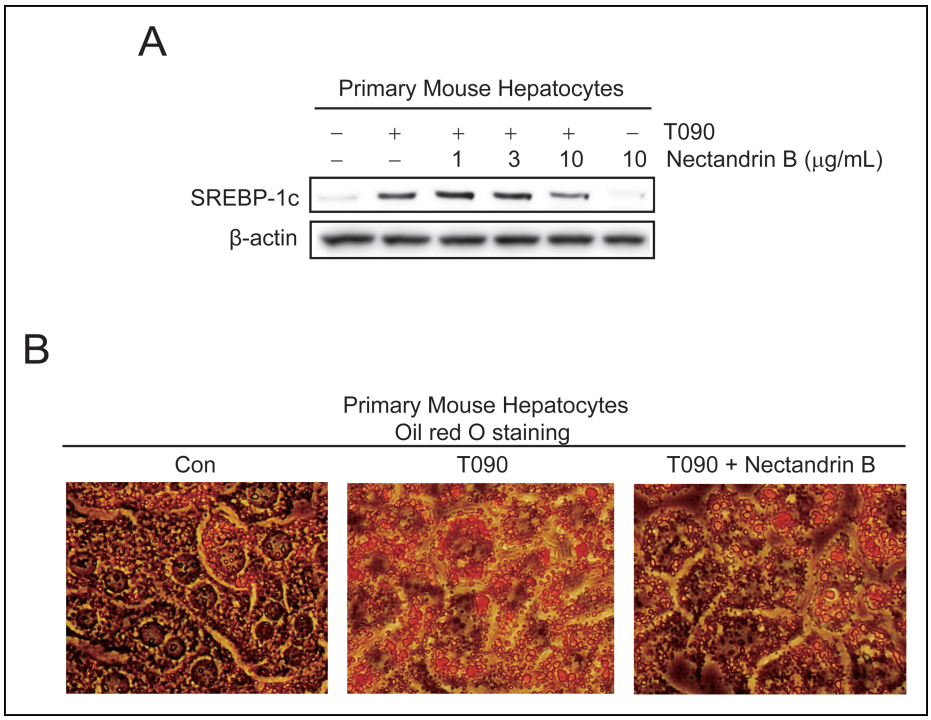

Fig. 4: Effect of nectandrin B on LXRα-induced SREBP-1c expression and lipid accumulation in primary mouse hepatocytes. (A) Cells were treated as described in the legend to Fig. 2A. (B) Cells were incubated with T0901317 (T090, 10 μM) for 24 h in the presence or absence of 10 μg/mL nectandrin B. Lipid droplets were detected by staining with Oil Red O. Representative images are shown.

tion with inflammation and fibrosis that elevates the risk of liver cirrhosis, an irreversible liver disease (Contos et al. 2004). Furthermore, NAFLD is referred to as hepatic manifestation of metabolic syndrome which is defined as the cluster of metabolic abnormalities including abdominal obesity, hyperglycemia, dyslipidemia, and hypertension (Asrih and Jornayvaz 2015; Alberti et al. 2005). Phytochemicals in plants are receiving growing attention because of their therapeutic potential for metabolic diseases (Soory 2009).

Nectandrin B is a tetrahydrofuran-type lignan isolated from nutmeg extract (Nguyen et al. 2010). In previous studies, nectandrin B has been shown to strongly activate AMPK, thus leading to vasorelaxation due to phosphorylation of endothelial nitric-oxide synthase, inhibition of neointima formation, and suppression of monocyte adhesion to endothelial cells (Hien et al. 2011; Ki et al. 2013; Hien et al. 2014). AMPK has been recognized as an effective therapeutic target for metabolic disorders including fatty liver, insulin resistance, and type 2 diabetes (Hardie 2011). It has been reported that the mixture of seven tetrahydrofuran-type lignans of nutmeg extract containing nectandrin B prevents the increases in body weight, epididymal fat mass, and the levels of blood glucose and LDL cholesterol in mice fed with high-fat diet (Nguyen et al. 2010). However, the molecular mechanisms for these anti-obesity and hypolipidemic effects have not been elucidated. We report here, for the first time, the protective effects of nectandrin B on the enhanced *de novo* fatty acid synthesis pathway and resultant lipid accumulation in hepatocytes.

In view of the important role of LXRα-SREBP-1c pathway in *de novo* lipogenesis and pathogenesis of fatty liver diseases, targeting this pathway would be therapeutically attractive for the treatment of NAFLD. In the present study, we demonstrated that nectandrin B completely represses the transcriptional activity of LXRα enhanced by a synthetic LXRα agonist. This inhibitory effect of nectandrin B was further supported by the suppression of mRNA expression of LXRα target genes, such as SREBP-1c and LXRα itself. Furthermore, we showed that nectandrin B also inhibits the increase in SREBP-1c expression promoted by insulin plus high glucose, two key endogenous stimuli for hepatic fatty acid synthesis. Several mechanisms have been identified to explain the activation of lipogenic pathway by either insulin or glucose, but commonly involve LXRα and SREBP-1c. Both insulin and high glucose activate SREBP-1c by stimulating its proteolytic cleavage and also increase the protein level of the precursor form of SREBP-1c (Hegarty et al. 2005; Guillet-Deniau et al. 2004). Moreover, LXRα is necessary for insulin-stimulated SREBP-1c induction (Chen et al. 2004) and glucose can act as an endogenous ligand for LXRα (Mitro et al. 2007). Collectively, we demonstrate here that nectandrin B has the ability to repress the induction of SREBP-1c precursor protein, presumably via the inhibition of LXRα activity.

The LXRα transcriptional activity is regulated by several kinases including protein kinase A, AMPK, p70 ribosomal S6 kinase-1, and c-Jun N-terminal kinase 1 (Yamamoto et al. 2007; Hwahng et al. 2009; Kim et al. 2010). AMPK suppresses LXRα activity by direct threonine-phosphorylation as demonstrated *in vitro* and in cell kinase assays (Hwahng et al. 2009). Several other mechanisms also contribute to the anti-lipogenic effects of AMPK (Lage et al. 2008). AMPK activation inactivates ACC1 by direct phosphorylation (Lage et al. 2008). Additionally, a recent paper suggested that AMPK directly phosphorylates SREBP-1c at Ser372, thus blocking the transcriptional activation of SREBP-1c target genes through the inhibition of SREBP-1c cleavage (Li et al. 2011). Taken together, the repression of LXRα-SREBP-1c pathway by nectandrin B can be explained at least in part by its stimulatory effect on AMPK activation. Finally, the suppressive effects of nectandrin B on the expression of major lipogenic genes, FAS and ACC1, and resultant fat accumulation in hepatocytes may be due to its inhibitory effect on the LXRα-SREBP-1c pathway.

In conclusion, we demonstrate that nectandrin B shows a potent stimulating effect on the AMPK activity in hepatocytes and has the ability to prevent hepatic lipogenesis and fat accumulation promoted by LXRα-SREBP-1c-mediated pathway. These

results may suggest the potential of nectandrin B as a promising therapeutic candidate for fatty liver disease.

4. Experimental

4.1. Materials

Antibodies against AMPK, phospho-AMPK, ACC, and phospho-ACC were obtained from Cell Signaling Technology (Beverly, MA). Anti-SREBP-1c antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). T0901317 was supplied by Calbiochem (San Diego, CA). Anti-β-actin antibody, GW3965, insulin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and other reagents were provided by Sigma-Aldrich Co. (St. Louis, MO). Nectandrin B was kindly provided by Korea Bioactive Natural Material Bank (KBNMB, Republic of Korea) at Seoul National University. It was isolated from nutmeg as described in the previous study (Nguyen et al. 2010) and its purity was confirmed to be > 95% by HPLC.

4.2. Cell culture

HepG2 cells were purchased from ATCC (Manassas, VA) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C.

4.3. Isolation of mouse hepatocytes

Mouse hepatocytes were isolated from C57BL/6 mice as previously described non-recirculating two-step perfusion method with a minor modification (Klaunig et al. 1981). Briefly, liver was perfused with Hank's balanced salt solution (HBSS) without calcium and magnesium at 37 °C for 5 min and then with DMEM containing 0.1% collagenase at a perfusion flow rate of 8 mL/min for 5 min. Next, the liver was excised and minced gently in DMEM containing 0.1% collagenase. The cell suspension was filtered through membrane filter and centrifuged at 50 g for 2 min at 4 °C. The pellet was resuspended with DMEM containing 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 10 nM dexamethasone and centrifuged at 50 g for 2 min at 4 °C. The viability of isolated hepatocytes was estimated to be above 90%. The isolated mouse hepatocytes were seeded at a density of 4×10^5 cells per well in 6-well plates. The culture medium was changed to serum free DMEM 4 h after plating. The next day, cells were used for further experiment.

4.4. Cell viability assay

HepG2 cells were seeded at a density of 5×10^4 cells per well in 48-well plates. The cells were incubated with 1-30 μg/mL nectandrin B for 24 h after overnight serum starvation and subsequently exposed to 0.5 mg/mL MTT for 1 h at 37 °C. Formazan crystals produced in each well were dissolved by adding 300 μl of dimethylsulfoxide after removal of the media. Absorbance was determined at 570 nm with an Infinite M200 PRO microplate reader (Tecan, Salzburg, Austria).

4.5. Immunoblot analysis

HepG2 cells were plated in 6-well plates and grown to reach 70%–80% confluency before serum starvation. Immunoblot analyses were conducted according to the procedures previously described (Kim et al. 2011).

4.6. Luciferase assay

Luciferase assay was performed according to the method previously described (Kim et al. 2011). Briefly, HepG2 cells were seeded in 6-well plates at a density of 4×10^5 cells/well. The next day, cells were transiently transfected with a pTK-CYP7A1-LXRE(×3)-LUC (1 μg) reporter gene construct containing three copies of an LXR response element (LXRE) sequence in rat *Cyp7a1* gene (Hwahng et al. 2009) and pCMV-LacZ using FuGENE (Roche, Nutley, NJ) in Opti-MEM (GIBCO BRL) for 3 h. The transfected cells were then incubated in Eagle's minimal essential medium (MEM) containing 1% FBS for 15 h and exposed to T0901317 in the presence or absence of nectandrin B for an additional 12 h.

4.7. Real-time PCR assay

Total RNA was extracted from HepG2 cells using Trizol (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's instructions. The cDNA was obtained by reverse-transcription of the isolated RNA (2 μg) with oligo-d$(T)_{16}$ primers in SureCycler 8800 (Agilent Technologies, Santa Clara, CA). Real-time PCR was conducted with LightCycler® 480 II instrument using LightCycler® 480 SYBR Green I Master solution according to the manufacturer's instructions (Roche Diagnostics, Indianapolis, IN). In addition, a melting curve analysis was performed to verify the accuracy of each amplicon. The mRNA level of interest was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The following primers were used; human SREBP-1 (sense: 5'-CGACATCGAAGACATGCTTCAG-3', antisense: 5'-GGAAGGCTTCAAGAGAGGAGC-3'); human LXRα (sense: 5'-GATCGAGGTGATGCTTCTGGAG-3', antisense: 5'-CCCTGCTTTGGCAAAGTCTTC-3'); human FAS (sense: 5'-GACATCGTCCATTCGTTTGTG-3', antisense: 5'-CGGATCACCTTCTTGAGCTCC-3'); human ACC1 (sense: 5'-GCTGCTCGGATCACTAGTGAA-3', antisense: 5'-TTCTGCTATCAGTCTGTCCAG-3') and human GAPDH (sense: 5'-GAAGGTGAAGGTCGGAGTC-3', antisense: 5'-GAAGATGGTGATGGGATTTC-3').

4.8. Oil Red O staining

After serum starvation, isolated mouse hepatocytes were exposed to 10 μM T0901317 for 24 h with 1 h pretreatment of vehicle or 10 μg/mL nectandrin B. The cells were washed with PBS and then fixed with 4% paraformaldehyde for 1 h. The cells were washed again with PBS, and stained with Oil Red O solution for 2 h at room temperature. The cells were then washed with DW and examined by microscopy (Nikon Eclipse Ti, Japan).

4.9. Statistical analysis

All data are reported as mean ± S.E. Statistically significant differences were assessed by the Student's *t*-test or one-way ANOVA with Bonferroni's multiple comparison test. A probability value of less than 0.05 was considered significant.

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References

Alberti KG, Zimmet P, Shaw J; IDF Epidemiology Task Force Consensus Group (2005) The metabolic syndrome-a new worldwide definition. Lancet 366: 1059–1062.

Asrih M, Jornayvaz FR (2015) Metabolic syndrome and nonalcoholic fatty liver disease: Is insulin resistance the link? Mol Cell Endocrinol pii: S0303–7207(15)00094–5 (in press).

Calkin AC, Tontonoz P (2012) Transcriptional integration of metabolism by the nuclear sterol-activated receptors LXR and FXR. Nat Rev Mol Cell Biol 13: 213–224.

Chen G, Liang G, Ou J, Goldstein JL, Brown MS (2004) Central role for liver X receptor in insulin-mediated activation of Srebp-1c transcription and stimulation of fatty acid synthesis in liver. Proc Natl Acad Sci USA 101: 11245–11250.

Contos MJ, Choudhury J, Mills AS, Sanyal AJ (2004) The histologic spectrum of nonalcoholic fatty liver disease. Clin Liver Dis 8: 481–500.

Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ (2005) Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest 115: 1343–1351.

Fabbrini E, Sullivan S, Klein S (2010) Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatology 51: 679–689.

Grover JK, Khandkar S, Vats V, Dhunnoo Y, Das D (2002) Pharmacological studies on Myristica fragrans – antidiarrheal, hypnotic, analgesic and hemodynamic (blood pressure) parameters. Methods Find Exp Clin Pharmacol 24: 675–680.

Guillet-Deniau I, Pichard AL, Koné A, Esnous C, Nieruchalski M, Girard J, Prip-Buus C (2004) Glucose induces de novo lipogenesis in rat muscle satellite cells through a sterol-regulatory-element-binding-protein-1c-dependent pathway. J Cell Sci 117: 1937–1944.

Hardie DG (2011) Sensing of energy and nutrients by AMP-activated protein kinase. Am J Clin Nutr 93: 891S–896S.

Hegarty BD, Bobard A, Hainault I, Ferré P, Bossard P, Foufelle F (2005) Distinct roles of insulin and liver X receptor in the induction and cleavage of sterol regulatory element-binding protein-1c. Proc Natl Acad Sci USA 102: 791–796.

Hien TT, Oh WK, Nguyen PH, Oh SJ, Lee MY, Kang KW (2011) Nectandrin B activates endothelial nitric-oxide synthase phosphorylation in endothelial cells: role of the AMP-activated protein kinase/estrogen

receptor α/phosphatidylinositol 3-kinase/Akt pathway. Mol Pharmacol 80: 1166–1178.

Hien TT, Ki SH, Yang JW, Oh WK, Kang KW (2014) Nectandrin B suppresses the expression of adhesion molecules in endothelial cells: Role of AMP-activated protein kinase activation. Food Chem Toxicol 66: 286–294.

Higuchi N, Kato M, Shundo Y, Tajiri H, Tanaka M, Yamashita N, Kohjima M, Kotoh K, Nakamuta M, Takayanagi R, Enjoji M (2008) Liver X receptor in cooperation with SREBP-1c is a major lipid synthesis regulator in nonalcoholic fatty liver disease. Hepatol Res 38: 1122–1129.

Hwahng SH, Ki SH, Bae EJ, Kim HE, Kim SG (2009) Role of adenosine monophosphate-activated protein kinase-p70 ribosomal S6 kinase-1 pathway in repression of liver X receptor-alpha-dependent lipogenic gene induction and hepatic steatosis by a novel class of dithiolethiones. Hepatology 49: 1913–1925.

Jakobsson T, Treuter E, Gustafsson JÅ, Steffensen KR (2012) Liver X receptor biology and pharmacology: new pathways, challenges and opportunities. Trends Pharmacol Sci 33: 394–404.

Joseph SB, Laffitte BA, Patel PH, Watson MA, Matsukuma KE, Walczak R, Collins JL, Osborne TF, Tontonoz P (2002) Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by liver X receptors. J Biol Chem 277: 11019–11025.

Ki SH, Lee JW, Lim SC, Hien TT, Im JH, Oh WK, Lee MY, Ji YH, Kim YG, Kang KW (2013) Protective effect of nectandrin B, a potent AMPK activator on neointima formation: inhibition of Pin1 expression through AMPK activation. Br J Pharmacol 168: 932–945.

Kim YM, Kim TH, Kim YW, Yang YM, Ryu da H, Hwang SJ, Lee JR, Kim SC, Kim SG (2010) Inhibition of liver X receptor-α-dependent hepatic steatosis by isoliquiritigenin, a licorice antioxidant flavonoid, as mediated by JNK1 inhibition. Free Radic Biol Med 49: 1722–1734.

Kim YM, Lim SC, Han CY, Kay HY, Cho IJ, Ki SH, Lee MY, Kwon HM, Lee CH, Kim SG (2011) G(alpha)12/13 induction of CYR61 in association with arteriosclerotic intimal hyperplasia: effect of sphingosine-1-phosphate. Arterioscler Thromb Vasc Biol 31: 861–869.

Klaunig JE, Goldblatt PJ, Hinton DE, Lipsky MM, Chacko J, Trump BF (1981) Mouse liver cell culture. I. Hepatocyte isolation. In vitro 17: 913–925.

Laffitte BA, Joseph SB, Walczak R, Pei L, Wilpitz DC, Collins JL, Tontonoz P (2001) Autoregulation of the human liver X receptor alpha promoter. Mol Cell Biol 21: 7558–7568.

Lage R, Diéguez C, Vidal-Puig A, López M (2008) AMPK: A metabolic gauge regulating whole-body energy homeostasis. Trends Mol Med 14: 539–549.

Li Y, Xu S, Mihaylova MM, Zheng B, Hou X, Jiang B, Park O, Luo Z, Lefai E, Shyy JY, Gao B, Wierzbicki M, Verbeuren TJ, Shaw RJ, Cohen RA, Zang M (2011) AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin resistant mice. Cell Metab 13: 376–388.

Lopez JM, Bennett MK, Sanchez HB, Rosenfeld JM, Osborne TE (1996) Sterol regulation of acetyl coenzyme A carboxylase: A mechanism for coordinate control of cellular lipid. Proc Natl Acad Sci USA 93: 1049–1053.

Mitro N, Mak PA, Vargas L, Godio C, Hampton E, Molteni V, Kreusch A, Saez E (2007) The nuclear receptor LXR is a glucose sensor. Nature 445: 219–223.

Moon YA, Liang G, Xie X, Frank-Kamenetsky M, Fitzgerald K, Koteliansky V, Brown MS, Goldstein JL, Horton JD (2012) The Scap/SREBP pathway is essential for developing diabetic fatty liver and carbohydrate-induced hypertriglyceridemia in animals. Cell Metab 15: 240–246.

Nguyen PH, Le TV, Kang HW, Chae J, Kim SK, Kwon KI, Seo DB, Lee SJ, Oh WK (2010) AMP-activated protein kinase (AMPK) activators from Myristica fragrans (nutmeg) and their anti-obesity effect. Bioorg Med Chem Lett 20: 4128–4131.

Olajide OA, Ajayi FF, Ekhelar AI, Awe SO, Makinde JM, Alada AR (1999) Biological effects of Myristica fragrans (nutmeg) extract. Phytother Res 13: 344–345.

Ram A, Lauria P, Gupta R, Sharma VN (1996) Hypolipidaemic effect of Myristica fragrans fruit extract in rabbits. J Ethnopharmacol 55: 49–53.

Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, Shan B, Brown MS, Goldstein JL, Mangelsdorf DJ (2000) Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. Genes Dev 14: 2819–2830.

Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, Schwendner S, Wang S, Thoolen M, Mangelsdorf DJ, Lustig KD, Shan B (2000) Role of LXRs in control of lipogenesis. Genes Dev 14: 2831–2838.

Sharma A, Mathur R, Dixit VP (1995) Prevention of hypercholesterolemia and atherosclerosis in rabbits after supplementation of Myristica fragrans seed extract. Indian J Physiol Pharmacol 39: 407–410.

Shimomura I, Shimano H, Korn BS, Bashmakov Y, Horton JD (1998) Nuclear sterol regulatory element–binding proteins activate genes responsible for the entire program of unsaturated fatty acid biosynthesis in transgenic mouse liver. J Biol Chem 273: 35299–35306.

Soory M (2009) Relevance of nutritional antioxidants in metabolic syndrome, ageing and cancer: potential for therapeutic targeting. Infect Disord Drug Targets 9: 400–414.

Van Gils C, Cox PA (1994) Ethnobotany of nutmeg in the spice islands. J Ethnopharmacol 42: 117–124.

Yamamoto T, Shimano H, Inoue N, Nakagawa Y, Matsuzaka T, Takahashi A, Yahagi N, Sone H, Suzuki H, Toyoshima H, Yamada N (2007) Protein kinase A suppresses sterol regulatory element-binding protein-1C expression via phosphorylation of liver X receptor in the liver. J Biol Chem 282: 11687–11695.