# The function of MGAT1 in alcohol-induced hepatic steatosis and treatment of hepatic steatosis via MGAT1 enzyme inhibition

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# The function of MGAT1 in alcohol-induced hepatic steatosis and treatment of hepatic steatosis via MGAT1 enzyme inhibition

Directed by Professor Jae-woo Kim

The Doctoral Dissertation submitted to the
Department of Medical Science,
The Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philisophy

Jung Hwan Yu

December 2015

This certifies that the Doctoral Dissertation of Jung Hwan Yu is approved.

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December 2015

# ACKNOWLEDGEMENT

그 동안 저를 지도해 주셨던 김재우 지도교수님 그리고 여러 생화학 분자생물학교실 교수님들, 같이 생활했던 랩원들에게 우선 감사의 말을 전합니다. 처음 생화학 교실에 와서 실험이란 것에 대해 잘 알지도 못하고, 낯선 환경 속에서 헤매던 저를 도와준 많은 사람들에게도 감사하다는 말을 전하고 싶습니다.

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정말 감사합니다.

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**ABSTRACT**

# The function of MGAT1 in alcohol-induced hepatic steatosis and treatment of hepatic steatosis via MGAT1 enzyme inhibition

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(Directed by Professor Jae-woo Kim)

Alcohol is the major cause of hepatic steatosis, and continuous consumption can lead to hepatic fibrosis, cirrhosis, and superimposed hepatocellular carcinoma. Hepatic steatosis is especially associated with metabolic diseases such as diabetes, and it is clearly present at the start of liver diseases. However, it is unclear how the alcohol is involved in fatty liver formation. Here, I evaluate the relationships between $NAD^+$/NADH ratio,

sirtuin1 (SIRT1) and peroxisome proliferator-activated receptor γ (PPARγ), to understand the mechanism of alcoholic fatty liver. In the course of ethanol consumption, $NAD^+$ was converted into NADH by alcohol dehydrogenase and aldehyde dehydrogenase, and subsequently SIRT1 level, which is regulated by $NAD^+$, decreased. Decreased SIRT1 activates PPARγ, a key transcription factor involved in lipogenesis, by increasing its acetylation. Activated PPARγ increased the expression of monoacylglycerol *O*-acyltransferase 1 (MGAT1) gene, known to have an important role in triacylglycerol synthesis, and PPARγ accelerated alcoholic hepatic steatosis. Moreover, MGAT1 knock-down significantly attenuated the alcohol-induced hepatic lipid accumulation. This result suggests that PPARγ and its target gene, MGAT1, have an important role in alcohol-induced fatty liver as well as non-alcoholic fatty liver.

To develop MGAT inhibitor, I set up a new screening method using Ellman's reagent, and performed the 1st screening using 2300 FDA-approved drugs. From these, 36 chemicals were shown to repress MGAT activity, and based on the structures of these chemicals, 300 additional chemicals with similar structures were screened. The final candidates came down to 20 substances. Of these, B1758 was administrated to ob/ob mice for 2 wk, showing a significant decrease in hepatic TG content and liver weight, suggesting that B1758 successfully suppresses hepatic steatosis. Taken together, the development of MGAT inhibitor may be a useful strategy to treat fatty liver

disease, which, if untreated, may induce chronic liver disease such as liver cirrhosis.

Key words: $NAD^+$/NADH ratio, SIRT1, PPARγ, MGAT1, alcoholic steatosis

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## I. INTRODUCTION

Alcoholic liver disease is associated with increased cardiovascular disease and diabetes, and it is a major cause of morbidity and mortality worldwide.[1] Accumulation of fat in the liver in response to alcohol consumption can lead to more harmful form of liver disease such as fibrosis, cirrhosis, and end stage liver injury. However, despite years of ongoing research, the mechanism by which alcohol consumption causes hepatic steatosis is complex and unclear.

Sirtuin1 (SIRT1) is a $NAD^+$- dependent deacetylase activated in response to fasting, caloric restriction, and physical exercise.[2] SIRT1 plays an important

role in hepatic lipid metabolism by modulating acetylation of transcription factor such as peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α).[3] Recent research reveals that SIRT1 signaling is also associated with alcoholic liver disease, and SIRT1 stimulation protects against alcohol-induced liver damage.[3,4] In adipocytes, SIRT1 promotes fat mobilization by repressing PPARγ, and it is associated with brown remodeling of white adipocytes by deacetylation of PPARγ.[5,6]

PPARγ is mainly present in adipose tissue, intestine, and macrophage, and it regulates fatty acid storage and glucose metabolism. Until now, the role of hepatic PPARγ has been underestimated because of its low expression in the normal liver. However, recent studies have revealed that PPARγ expression is increased in a mouse model of obesity and plays a critical role in hepatic steatosis by regulating the expression of lipogenic genes. Additionally, hepatic PPARγ expression is associated with TG synthesis and lipid accumulation.[7,8]

MGAT is an enzyme that catalyzes the synthesis of diacylglycerol (DAG), a precursor of triacylglycerol (TG).[9] There are two subtypes of MGATs (MGAT1 and MGAT2) in mice. MGAT1 is well known the role in liver, and MGAT2 is considered to have a role in intestine.[9,10] Previously, I identified MGAT1 as a novel target gene of PPARγ and reported the beneficial metabolic effects of hepatic MGAT1 suppression in non-alcoholic fatty liver disease

(NAFLD).[8] Subsequently, other groups have also reported that hepatic MGAT1 has an important role in hepatic steatosis.[11,12] Although these studies are restricted to non-alcoholic heaptic steatosis, our study implicates a possibility that MGAT1 enzyme has an important role with PPARγ acitivation in alcohol-induced hepatic steatosis.

The pathogenesis of alcoholic hepatic steatosis is still unclear, but recent studies indicated that ethanol increases fatty acid synthesis in hepatocytes by regulating lipid metabolism associated transcription factor such as sterol regulatory element-binding protein 1c (SREBP1c) and carbohydrate-reponsive element-binding protein (ChREBP); these transcription factors promote fatty acid synthesis via up-regulation of lipogenic genes.[1,13] In addition, the endoplasmic reticulum response to cell stress, endocannabinoids, tumor necrosis factor –α, and LPS signaling via Toll-like receptor are known to be associated with alcohol induced hepatic steatosis, directly or indirectly.[14-16]

In this study, I investigated that the cellular and molecular mechanism of alcoholic hepatic steatosis, and we found that PPARγ activity was involved in alcohol-induced hepatic steatosis. Furthermore, I could observe that the inhibition of MGAT1 efficiently attenuated the lipid accumulation by alcohol consumption in liver. As a result, I suggest that PPARγ is a major regulator of alcohol-induced hepatic steatisis and the development of MGAT1 inhibitor

could be a very useful therapeutic target of alcoholic or non-alcoholic hepatic steatosis.

## II. MATERIALS AND METHODS

### 1. Mice and Diet

Male C57BL/6J mice were purchased from Japan SLC (Shiwuoka, Japan). The animals were maintained in a temperature-controlled room (22°C) on a 12:12-h light–dark cycle. Seven- or 8-wk-old mice were fed a Lieber-DeCarli liquid diet (Dyets, Bethlehem, PA, USA) containing 1 Kcal/ml, of which 18% was derived from protein, 35% of fat, and either 47% from carbohydrate (control diet) or 11% from carbohydrate and 36% from ethanol (alcohol diet) for up to 4 wk. Ethanol was introduced gradually by increasing the content by 9% of its total calorie-intake until the mice were consuming a diet containing 27% ethanol and was then continued for three more weeks. Mice were paired-fed, and body weight and food intake were monitored daily. Body weight was measured once a week. Adenovirus injection ($2 \times 10^9$ pfu) through tail vein was done at 2 or 3 wk of liquid diet period to suit each experiment. At the end of experiment, mice were sacrificed and liver tissues and blood samples were collected. Male SIRT1 transgenic mice were purchased from *Jackson* Laboratory (Bar Harbor, Maine, USA) and genotyping by PCR was performed as described previously.[17] Binge drinking was done modifying the protocol recently reported by Dr. Postic.[13] Twelve-week-old male C57BL/6J mice were

fasted for 4 hr before receiving 2 gavages of equivalent calories of ethanol at 3.5 g/kg or dexrin-maltose (DM; MP Biomedicals, USA). Mice were kept on a heat-pad for the experiment to prevent hypothermia. Then, mice were sacrificed 6 hr later. All procedures were approved by the Committee on Animal Investigations of Yonsei University.

**2. RNA Isolation and Analysis of Gene Expression by Quantitative RT-PCR**

Total RNA was isolated using TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. First-strand cDNA synthesis from 5 μg total RNA was performed using SuperScript III reverse transcriptase (Invitrogen) primed by random hexamer primers. Real-time qPCR was performed using SYBR Green Master mix (Applied Biosystems) in a total volume of 20 μL with a Step One instrument (Applied Biosystems). Rplp0 gene was also measured as an invariant control. The primer sequences used in real-time qPCR are as follows: PPARγ, 5′-CTCTG GGAGA TTCTC CTGTT-3′, 5′-GGTGG GCCAG AATGG CATCT-3′; SREBP1c, 5′-GGAGC CATGG ATTGC ACATT-3′, 5′-GGCCC GGGAA GTCAC TGT-3′; ChREBP, 5′-CCTCA CTTCA CTGTG CCTCA-3′, 5′-ACAGG GGTTG TTGTC TCTGG-3′; aP2/422, 5′-TCTCC AGTGA AAACT TCGAT-3′, 5′-TACGC TGATG

ATCAT GTTG-3′; FSP27, 5′-TCCAG GACAT CTTGA AACTT-3′, 5′-GGCTT GCAAG TATTC TTCTG T-3′; Cd36, 5′-TGCAC CACAT ATCTA CCAAA-3′, 5′-TTGTA ACCCC ACAAG AGTTC-3′; FAS, 5′- AAGCC GTTGG GAGTG AAAGT-3′, 5′-CAATC TGGAT GGCAG TGAGG-3′; MGAT1, 5′-CTGGT TCTGT TTCCC GTTGT-3′, 5′-TGGGT CAAGG CCATC TTAAC-3′; L-PK, 5′-CCGAG ATACG CACTG GAGTC-3′, 5′-GTGGT AGTCC ACCCA CACTG-3′; SCD1, 5′-TTCTC AGAAA CACAC GCCGA-3′, 5′-AGCTT CTCGG CTTTC AGGTC-3′; GPAT, 5′-TCCTA GCTCG CGATT TCGAC-3′, 5′-ATCTT TCCTG CTCGT GTGGG-3′; Elovl6, 5′-TGCTG ATGGG CTGTG TCATT-3′, 5′-GGAGT AGCAC TGGTC GTTGT-3′; G0S2, 5′-AAAGT GTGCA GGAGC TGATC-3′, 5′-GGACT GCTGT TCACA CGCTT-3′ and Rplp0, 5′-GCAGG TGTTT GACAA CGGCA G-3′, 5′-GATGA TGGAG TGTGG CACCG A-3′.

**3. Western Blot Analysis**

For protein preparation from liver tissues, mouse livers (50 mg) were placed in a glass homogenizer containing 1 mL Pro-Prep Protein Extraction Solution (Intron Biotechnology). Tissue lysates were separated by electrophoresis on SDS/PAGE gels. Gels were transferred onto nitrocellulose membranes and the blots were incubated with blocking buffer [5% (wt/vol)

nonfat dried milk in TBST] before incubation with antibodies. Primary antibodies used for blotting were anti-SIRT1 (Santa Cruz Biotechnology, Dalaware Avenue, CA, USA), anti-PPARγ, anti-acetylated lysine (Cell Signaling, Danvers, MA, USA) and anti–β-actin. Bands were detected with anti-rabbit or anti-mouse IgG conjugated with horseradish peroxidase (Pierce, Rockford, IL, USA), using the ECL-PLUS detection system (Amersham Biosciences, Piscataway, NJ, USA).

**4. Primary Hepatocyte Isolation**

Primary mouse hepatocytes were isolated by the two-step collagenase perfusion method from the livers of male C57BL/6 (8 wk old) as previously described.[18] Hepatocytes were plated on six-well dishes at $1.0 \times 10^6$ cells per well and incubated for 12 hr in DMEM containing 10%(vol/vol) FBS to allow cells to attach. Cell counts and viability (Adam cell counter; Digital Bio, Seoul, Korea) were confirmed before use; viability was routinely >85%. After attachment, cells were infected with PPARγ and SIRT1 adenoviruses.

**5. Preparation of Recombinant Adenovirus**

Murine PPARγ and SIRT1 cDNAs were cloned into pcDNA3 vector or FLAG-tagged pcDNA3, respectively. Recombinant adenovirus expressing PPARγ and MGAT1-FLAG and ad-shRNA for MGAT1 were prepared. All viruses were propagated in 293 cells and purified by CsCl density purification, dissolved in 1x HBSS (Invitrogen, Carlsbad, CA, USA) and stored at -70°C. The multiplicity of infection (MOI) was calculated from viral particle numbers. Recombinant adenovirus containing the GFP gene or Ad-US control RNAi was used as a control.

**6. Triglyceride (TG) and Cholesterol Assay in the Liver**

Liver extracts (200 mg) were prepared by homogenization in chloroform:methanol (2:1) with final volume of 4 ml. The homogenate was incubated with vortexing for 10 min, and then centrifuged at 4°C for 10 min. TG concentrations were determined using 25 ul of extract in a commercial colorimetric assay (Thermo Scientific, Waltham, MA, USA). Samples and standards were vortexed and incubated at 37°C for 30 min, and TG level were calculated from measurements of absorbance at 500 nm and expressed as mg TG/g liver wet weight.

**7. Transfection and Luciferase assay**

HepG2 cells were transfected with 0.8 μg pGL3-MGATs promoter plasmid, 0.1 μg expression vector plasmid, and 10 ng pRL-CMV (Promega, Madison, WI, USA), using Lipofectamine (Invitrogen), according to the manufacturer's instructions. After 24 hr, cells were harvested and the luciferase activities were measured using the Dual Luciferase Reporter Assay System (Promega), according to the manufacturer's instructions. Firefly luciferase activities were standardized to Renilla activities.

**8. $NAD^+$/NADH assay**

$NAD^+$/NADH ratio was measured using $NAD^+$/NADH assay kits from Abcam (Cambridege, MA, USA) according to manufacturer's instructions. 20mg of liver tissues and $2x10^5$cells per well (6well plate) were used for analysis. Cells and Tissues were extracted with $NAD^+$/NADH extraction buffer. Total $NAD^+$ and NADH were detected following the instructions in a 96-well plate. Absorbance was measured at 450 nm using multidetection reader (VERSA max, Molecular Devices).

**9. Immunocytochemistry**

Primary mouse hepatocytes were isolated from the livers of WT or SIRT Tg mice (10 wk old, male), and then they were transfected with Ad-GFP or Ad-PPARγ. At the indicated times, cells were washed with PBS, fixed in 4% formaldehyde for 15 min, permeablized with 0.2% Triton X-100 for 20 min on ice, and then blocked in 3% bovine serum albumin in PBS for 1 hr. Cells were then incubated in a blocking solution containing ADRP antibody (1:200 dilution) for 12 hr, followed by the secondary antibody (anti-mouse IgG-fluorescein isothocyanate) for 2 hr. The cells were mounted in 4',6-diamidino-2-phenylindole. Cells were then visualized with Confocal Laser Scanning Microscope (Carl Zeiss, Thornwood, NY, USA).

**10. Immunoprecipitation and PPARγ acetylation**

For immunoprecipitation, we lysed tissues using passive lysis buffer (50 mM Tric-HCl, pH 7.4, 1% NP-40, 0.25% Sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, and protease inhibitor cocktail). Lysated proteins were immunoprecipiated with anti-PPARγ (E8, Santa Cruz Biotechnology), collected by adding protein A/G plus agarose beads (Santa Cruz Biotechnology), and washed three times with lysis buffer. Acetylated levels on immunoprecipatated

proteins were assessed using anti-acetylated lysine antibody (Cell Signaling). Western blots for PPARγ were also performed to determine the total protein amount.

**11. Statistical analysis**

All results are expressed as mean ± SD with n representing the number of analyzed mice. Statistical comparisons of groups were made using an unpaired Student's *t* test. $P<0.05$ was considered statistically significant.

## III. RESULT

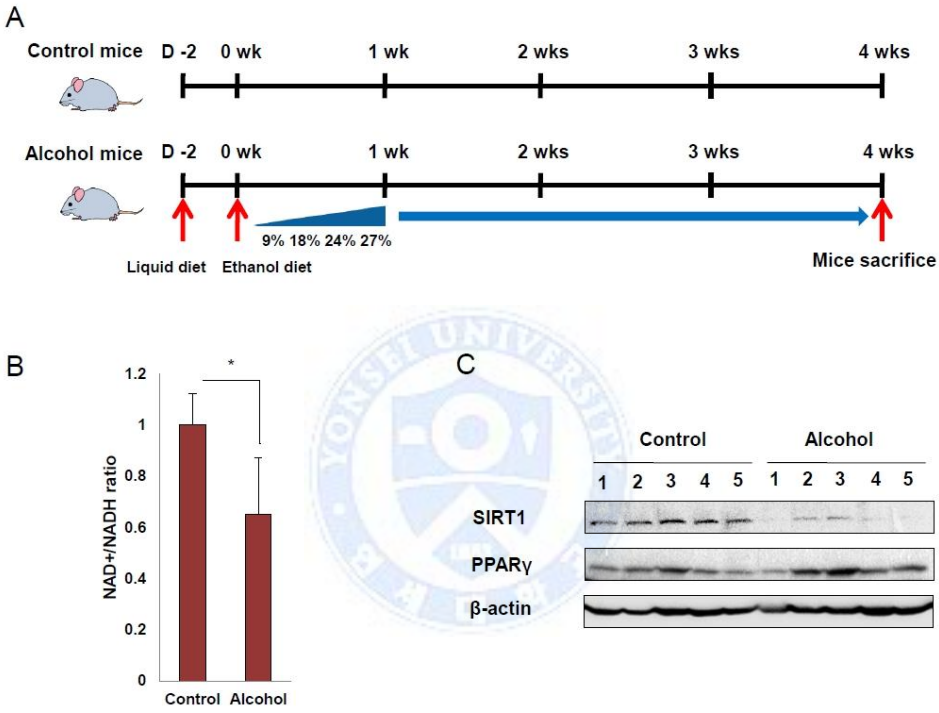
### 1. PPARγ and MGAT1 in alcohol-induced hepatic steatosis

#### A. Ethanol decreased the $NAD^+$/NADH ratio and SIRT1 expression

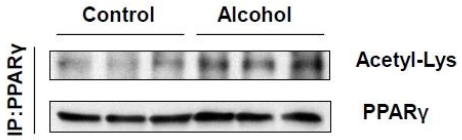
To study the alcohol metabolism, we used C57BL/6 (B6) mice which are widely used in metabolic disease research. Male B6 mice were pair fed Liber-Decarli liquid diet with or without (27% of total calorie) ethanol for 4 wk (Fig. 1A). Exposure to Liber-Decali ethanol diet for 4 wk resulted in a significant increase in hepatic TG contents and liver weight, which means had ethanol successfully induced hepatic steatosis. In ethanol fed mice group, $NAD^+$/NADH ratio decreased (Fig. 1B), and NAD dependent deacetylase sirtuin 1 (SIRT1) protein level also decreased (Fig. 1C). PPARγ protein level, in some cases, seemed to increase, but not so clearly in this study. However, the acetylation of PPARγ increased in ethanol mice group (Fig. 1D). The gene expression of PPARγ and SREBP1c, known to play an important role pathophysiology of alcoholic hepatic steatosis, had increased in alcohol diet fed mice. Furthermore, the expression of PPARγ target genes (MGAT1, CD36, and G0S2) and SREBP1c target genes (L-PK, SCD1, GPAT, Elvol6, and FAS) had also increased (Fig. 1E, F).

There are seven types of sirtuins currently known, and we have checked the mRNA expression of other sirtuins affected by ethanol diet. As shown in Fig.

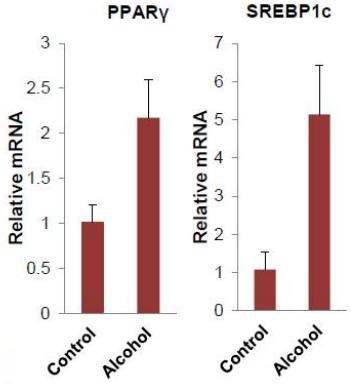
3, mRNA expression levels of all sirtuin types were generally reduced in alcohol mice group. At this study, I focused on sirtuin 1 to evaluate the mechanism of alcohol-induced hepatic steatosis.


A
Control mice
D -2
0 wk
1 wk
2 wks
3 wks
4 wks
Alcohol mice
D -2
0 wk
1 wk
2 wks
3 wks
4 wks
9% 18% 24% 27%
Liquid diet
Ethanol diet
Mice sacrifice
B
NAD+/NADH ratio
1.2
1
0.8
0.6
0.4
0.2
0
*
Control
Alcohol
C
Control
Alcohol
1 2 3 4 5 1 2 3 4 5
SIRT1
PPARγ
β-actin

D


Control
Alcohol
IP:PPARγ
Acetyl-Lys
PPARγ

E


PPARγ
SREBP1c
Relative mRNA
Relative mRNA
Control
Alcohol
Control
Alcohol

F

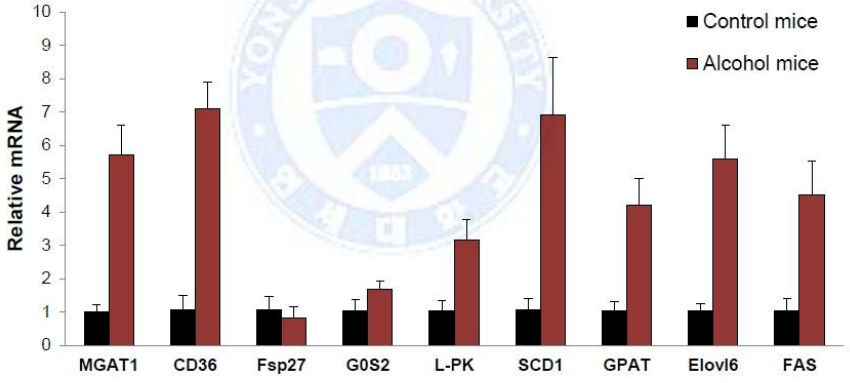
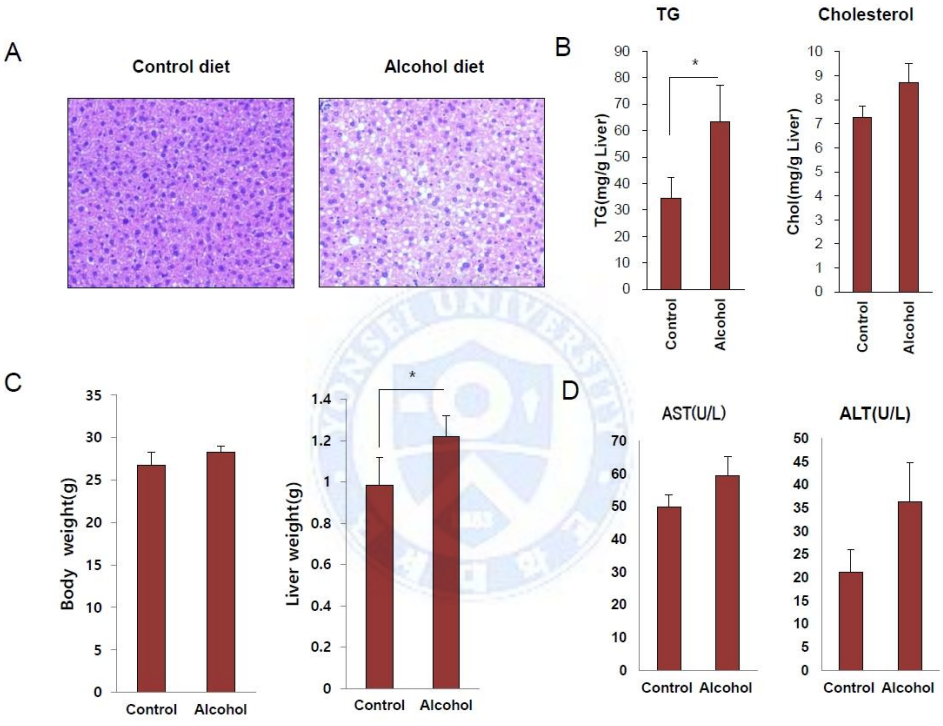

Control mice
Alcohol mice
Relative mRNA
MGAT1
CD36
Fsp27
G0S2
L-PK
SCD1
GPAT
Elovl6
FAS

**Fig. 1. Ethanol decreases the $NAD^+$/NADH ratio and SIRT1 expression in mice.** (A) Schedule of ethanol and control diet in mice experiment. (B) $NAD^+$/NADH ratio checked in the livers of control diet and ethanol diet-fed mice. (n=6/group) (C, D) Western blot analysis of SIRT1, PPARγ and β-actin, and PPARγ acetylation in control and ethanol diet-fed mice. (E, F) PPARγ and SREBP1c expression level and their target genes in control and ethanol diet-fed mice. (n=6-8/group) Data in B represent the mean ± SD. *P < 0.05.


A
Control diet
Alcohol diet
B
TG
TG(mg/g Liver)
Control
Alcohol
Cholesterol
Chol(mg/g Liver)
Control
Alcohol
C
Body weight(g)
Control
Alcohol
Liver weight(g)
Control
Alcohol
D
AST(U/L)
Control
Alcohol
ALT(U/L)
Control
Alcohol

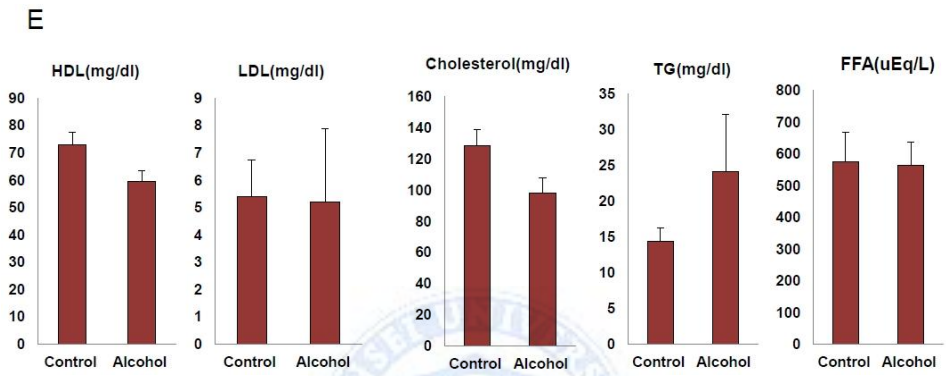



**Fig. 2. Metabolic parameters in alcohol-induced hepatic steatosis.** (A) H&E staining performed on liver sections from mice as shown. (B) Hepatic TG and cholesterol contents. (C) Body weight and liver weight. (D) AST and ALT level in mice blood sample. (E) Blood parameters in control and alcohol-fed mice. Data in B, C represent the mean ± SD. *P < 0.05.

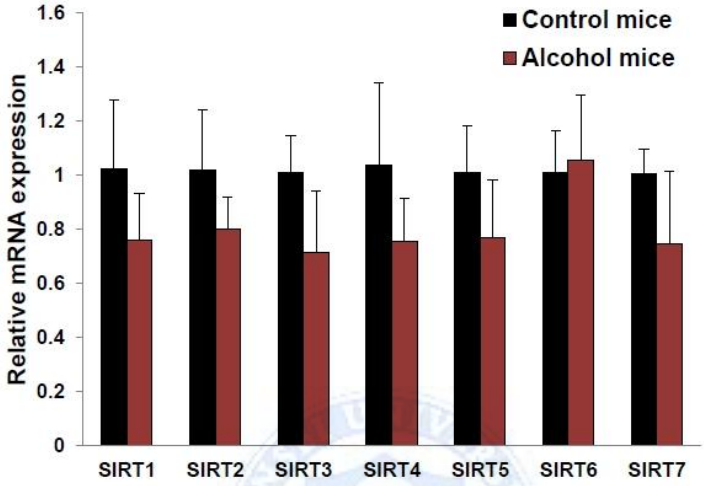
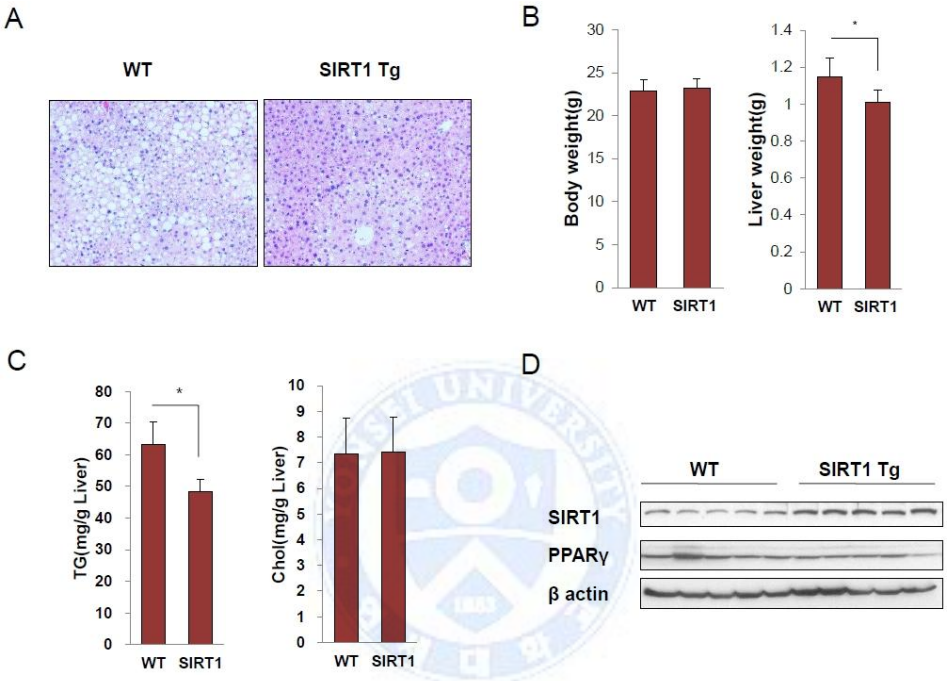



**Fig. 3. mRNA expression levels of sirtuin subtypes in alcohol-fed mice.** mRNA expression levels of currently known sirtuin subtypes were checked. mRNA expression levels of all sirtuin types were generally reduced in alcohol-fed mice group. Data represent the mean ± SD.

**B. SIRT1 protects liver from alcohol-induced hepatic steatosis**

We used SIRT1 Tg mice to evaluate the effect of SIRT1 on ethanol metabolism. WT and SIRT1 Tg mice were pair fed Liber-Decarli liquid diet with ethanol (27% of total calorie) for 4 wk. During the feeding period, food intake was similar and the ethanol feeding had no apparent effect on the health status of both the WT or SIRT1 Tg mice. SIRT1 Tg mice showed protective effect on ethanol-induced hepatic steatosis (Fig. 4A). The body weight was not different; however, the liver weight decreased in SIRT1 Tg mice group. Moreover, liver TG contents also decreased significantly (Fig. 4C). In SIRT1 Tg mice, PPARγ level was not changed, but the acetylation of PPARγ tended to decrease (Fig. 4D, E). Furthermore, the expressions of PPARγ target genes such as MGAT1, aP2, and CD36 also decreased (Fig. 4F). This indicates that PPARγ activity was decreased. In SIRT1 TG mice, AST and ALT levels, representing liver inflammation, were also decreased (Fig. 4G). Similar results were shown in SIRT1 overexpression experiment using adenovirus (Fig. 5A-E). Overall, I can observe that SIRT1 has a protective and beneficial role in alcohol-induced hepatic steatosis.


A
WT
SIRT1 Tg
B
Body weight(g)
30
25
20
15
10
5
0
WT
SIRT1
Liver weight(g)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
WT
SIRT1
C
TG(mg/g Liver)
80
70
60
50
40
30
20
10
0
*
WT
SIRT1
Chol(mg/g Liver)
10
9
8
7
6
5
4
3
2
1
0
WT
SIRT1
D
WT
SIRT1 Tg
SIRT1
PPARγ
β actin

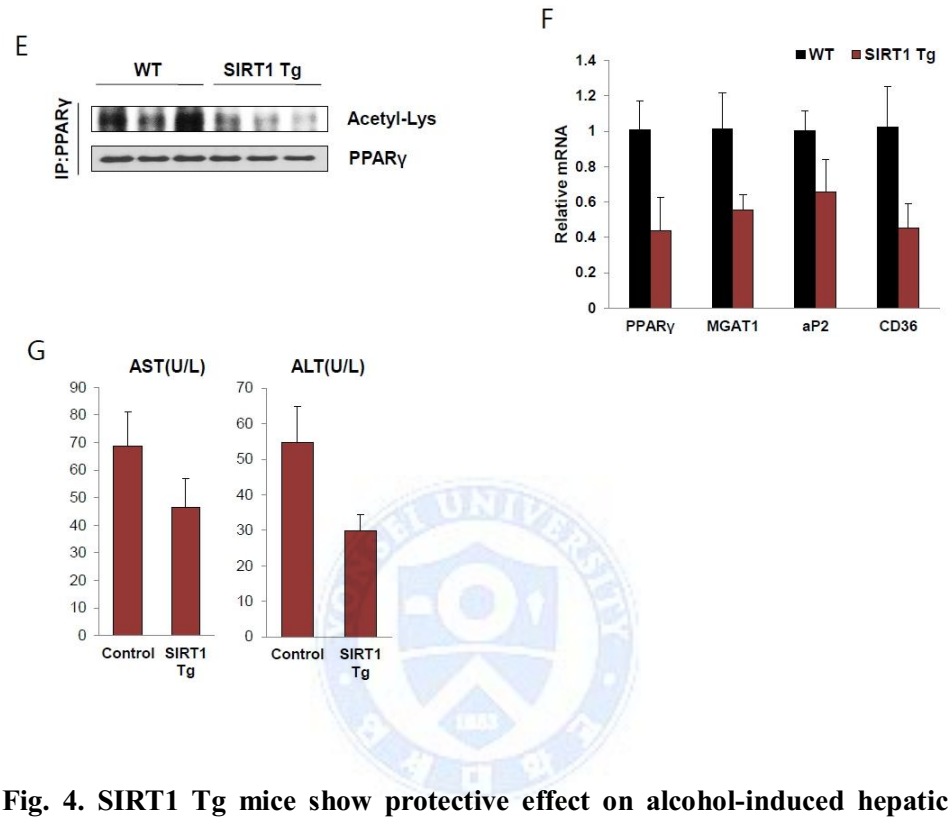
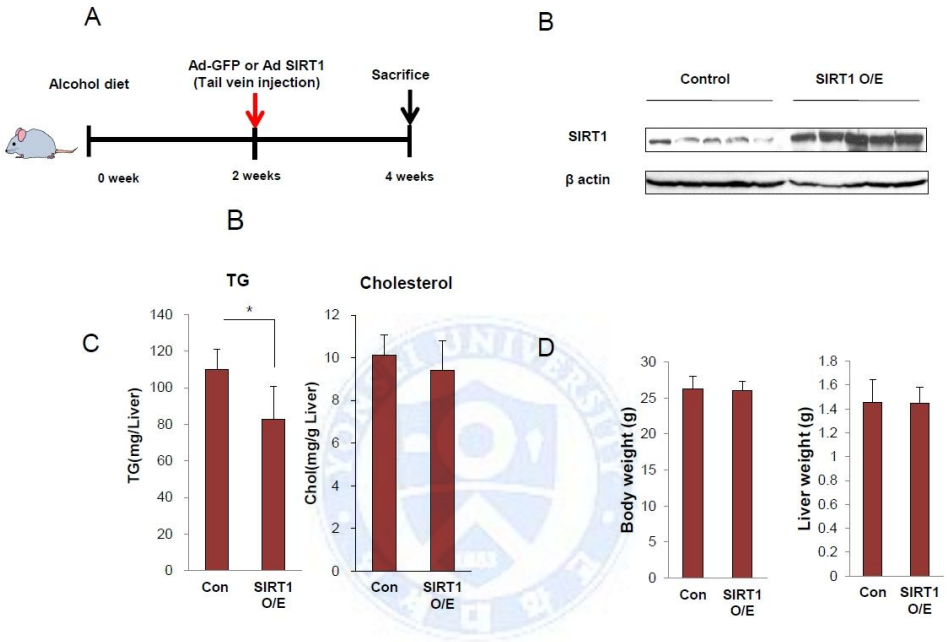



**Fig. 4. SIRT1 Tg mice show protective effect on alcohol-induced hepatic steatosis.** (A) H&E staining performed on liver sections from mice. (B) Body weights and liver weights in WT and SIRT1 Tg mice. (C) TG and cholesterol contents in livers. (D, E) Western blot analysis and PPARγ acetylation in WT and SIRT1 Tg mice. (F) Real-time PCR analysis of PPARγ and its target genes in liver. (G) AST and ALT levels in blood samples. (n=6/ group) Data in B and C represent the mean ± SD. *P < 0.05.


A
Alcohol diet
Ad-GFP or Ad SIRT1
(Tail vein injection)
Sacrifice
0 week
2 weeks
4 weeks
B
Control
SIRT1 O/E
SIRT1
β actin
B
TG
Cholesterol
C
TG(mg/Liver)
Chol(mg/g Liver)
Con
SIRT1 O/E
D
Body weight (g)
Liver weight (g)

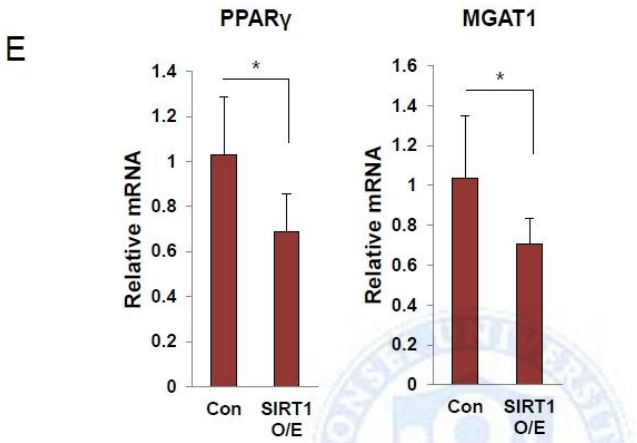
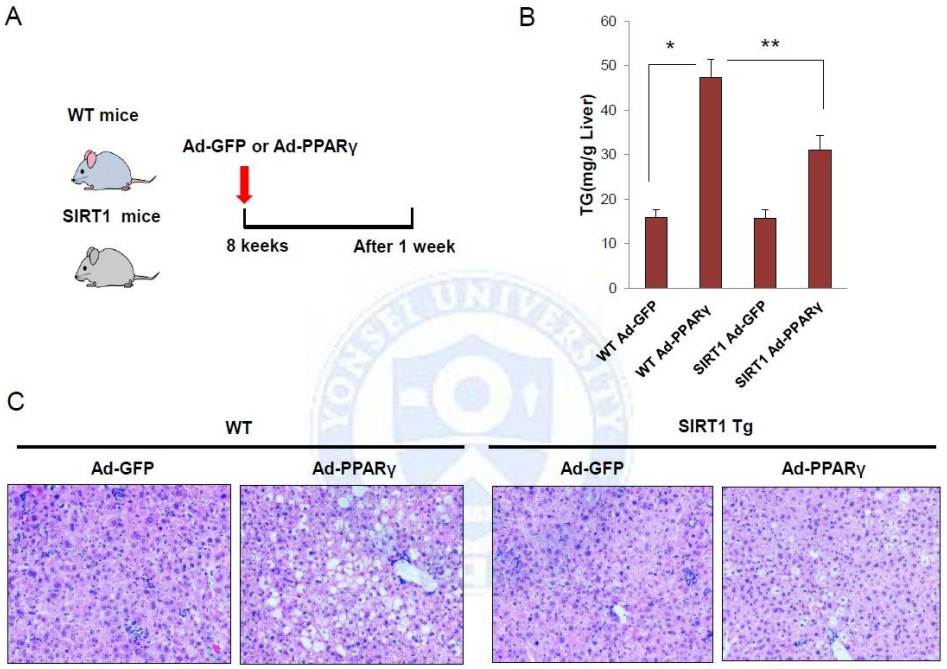


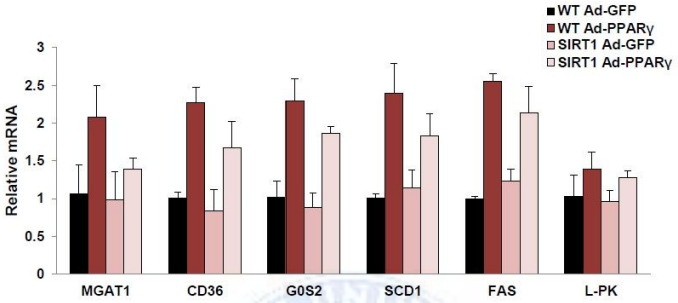

**Fig. 5. The effect of SIRT1 overexpression via adenovirus injection in alcohol-induced hepatic steatosis.** (A) Schedule of SIRT1 adenovirus injection experiment. (B) Western blot analysis showing the SIRT1 expression in Ad-GFP and Ad-SIRT1 injected mice. (C) Hepatic TG and cholesterol contents. (D) Body weight and liver weight. (E) Real-time PCR analysis showing PPARγ, and MGAT1. n=5. Data in C, E represent the mean ± SD. *P < 0.05

**C. PPARγ is an important transcription factor for TG synthesis, and SIRT1 attenuates PPARγ-induced hepatic steatosis**

To verify the role of PPARγ in the fatty liver, we injected adenoviral PPARγ in WT and SIRT1 Tg mice via tail veins, resulting in overexpression of PPARγ. Mice at 8 wk old were injected with Ad-PPARγ or Ad-GFP, fed with chow diet for 1 wk, and then were sacrificed (Fig. 6A). Ad-PPARγ-injected mice showed higher levels of hepatic TG than control Ad-GFP mice (Fig. 6B). Histological analysis revealed the presence of numerous fat droplets in the livers of the Ad-PPARγ injected mice (Fig. 6C). However, when Ad-PPARγ was injected, SIRT1 TG mice showed lower level of hepatic TG than WT mice did (Fig. 6B, C). PPARγ overexpression resulted in an induction of several PPARγ targets and lipogenic genes, but the increase in gene expression was attenuated in SRIT1 TG mice (Fig 6D). We also analyzed the effect of PPARγ overexpression in primary mouse hepatocytes. These cells were subjected to immunocytochemistry using anti-ADRP antibody, which is PPARγ target gene and localizes to the lipid droplet. PPARγ overexpression resulted in increased ADRP expression in primary hepatocyte, however the increase was significantly attenuated in cells isolated from SIRT1 TG mice (Fig. 6E). As this result suggests, PPARγ is a major regulator in hepatic TG synthesis and SIRT1 inhibits PPARγ-induced hepatic steatosis.


A
WT mice
Ad-GFP or Ad-PPARγ
SIRT1 mice
8 keeks
After 1 week
B
TG(mg/g Liver)
60
50
40
30
20
10
0
*
**
WT Ad-GFP
WT Ad-PPARγ
SIRT1 Ad-GFP
SIRT1 Ad-PPARγ
C
WT
SIRT1 Tg
Ad-GFP
Ad-PPARγ
Ad-GFP
Ad-PPARγ

D


WT Ad-GFP
WT Ad-PPARγ
SIRT1 Ad-GFP
SIRT1 Ad-PPARγ
Relative mRNA
3
2.5
2
1.5
1
0.5
0
MGAT1
CD36
G0S2
SCD1
FAS
L-PK

E

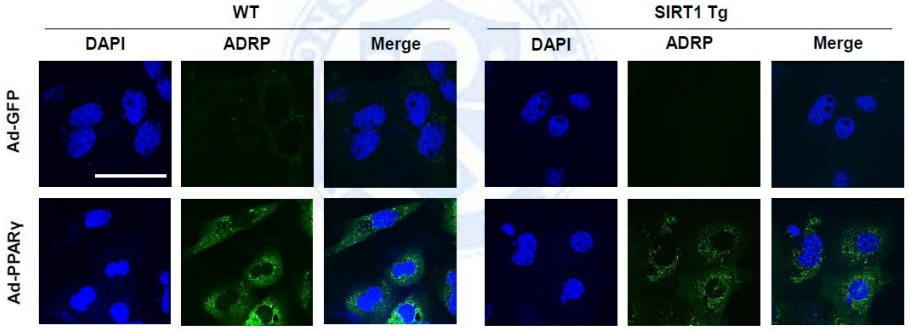
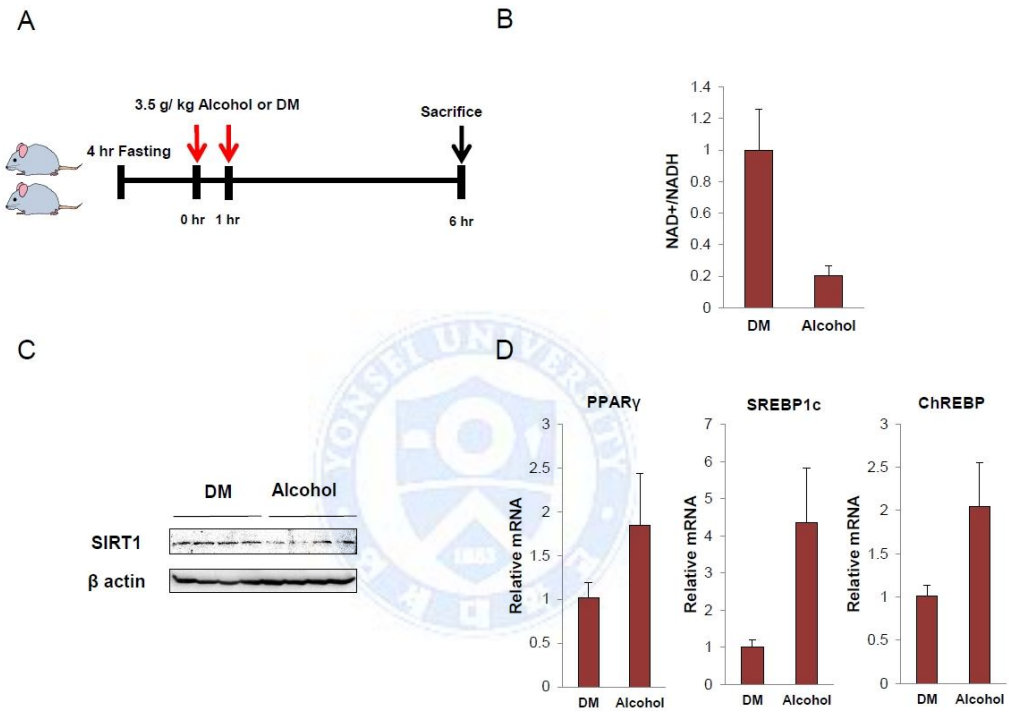

WT
SIRT1 Tg
DAPI
ADRP
Merge
DAPI
ADRP
Merge
Ad-GFP
Ad-PPARγ

**Fig. 6. The effect of PPARγ overexpression in WT and SIRT1 Tg mice.** (A) Schedule of PPAR overexpression experiment. WT and SIRT1 Tg mice were injected with Ad-PPARγ or an Ad-GFP, fed with chow diet for 1 wk, and then were sacrificed. (B) Hepatic TG content determined in WT and SIRT1 Tg mice infected with Ad-GFP or Ad-PPARγ. (C) H&E staining performed on liver section of WT and SIRT1 Tg mice. (D) Real-time PCR analysis of PPARγ target genes and lipogenic genes in WT and SIRT1 Tg mice. (E) Immunofluorescence staining for ADRP (green) of mouse primary hepatocyte. Nuclei were stained with DAPI and fluorescence was visualized by confocal microscopy. (Scale bar=50μm) Data in B represent the mean ± SD. *P < 0.05.

**D. Ethanol metabolism in binge drinking**

Binge drinking is the act of consuming heavy amount of alcohol in a short time period. Recently, the role of ChREBP in binge drinking was reported.[13] In this report, acute alcohol drinking affected the activity of ChREBP by regulating its acetylation. Therefore, we explored the role of PPARγ and the early response to alcohol consumption through binge drinking mice model. Twelve week-old male C57BL/6J mice were fasted for 4 hr before receiving 2 doses of gavages with equivalent calories of dextrin-maltose (DM) or EtOH at 3.5 g/kg (Fig. 7A). The $NAD^+$/NADH ratio definitely decreased in the binge drinking group compared to control mice group (Fig. 7B). SIRT1 level was also decreased after binge drinking (Fig. 7C). mRNA expressions of SREBP1c, ChREBP, and PPARγ significantly increased in livers of EtOH mice group compared to DM mice group (Fig. 7D). In addition, the expression of SREBP1c and ChREBP target genes such as SCD1, L-PK, and FAS was increased. However, most PPARγ target genes remained at control level, despite the increase of PPARγ expression (Fig. 7E). Thus, we hypothesized that binge drinking initially affects SREBP1c or ChREBP, and eventually PPARγ target genes are affected during lipid accumulation.


A
3.5 g/ kg Alcohol or DM
Sacrifice
4 hr Fasting
0 hr
1 hr
6 hr
B
NAD+/NADH
1.4
1.2
1
0.8
0.6
0.4
0.2
0
DM
Alcohol
C
DM
Alcohol
SIRT1
β actin
D
PPARγ
SREBP1c
ChREBP
Relative mRNA
3
2.5
2
1.5
1
0.5
0
7
6
5
4
3
2
1
0
DM
Alcohol

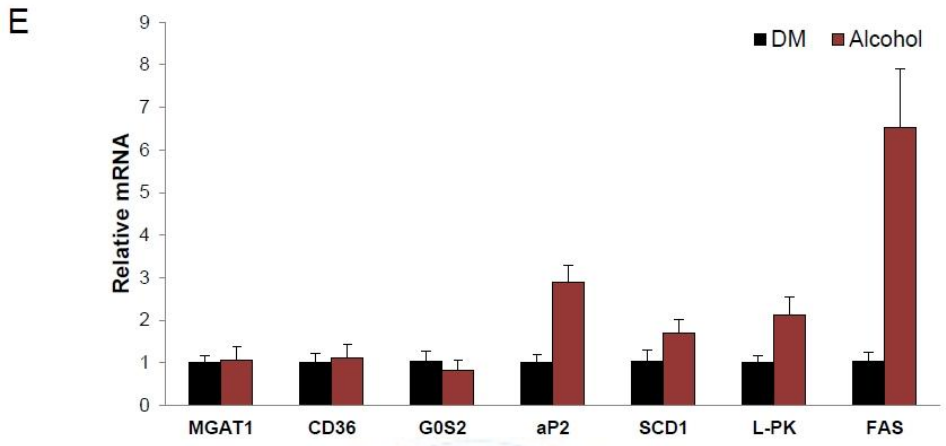
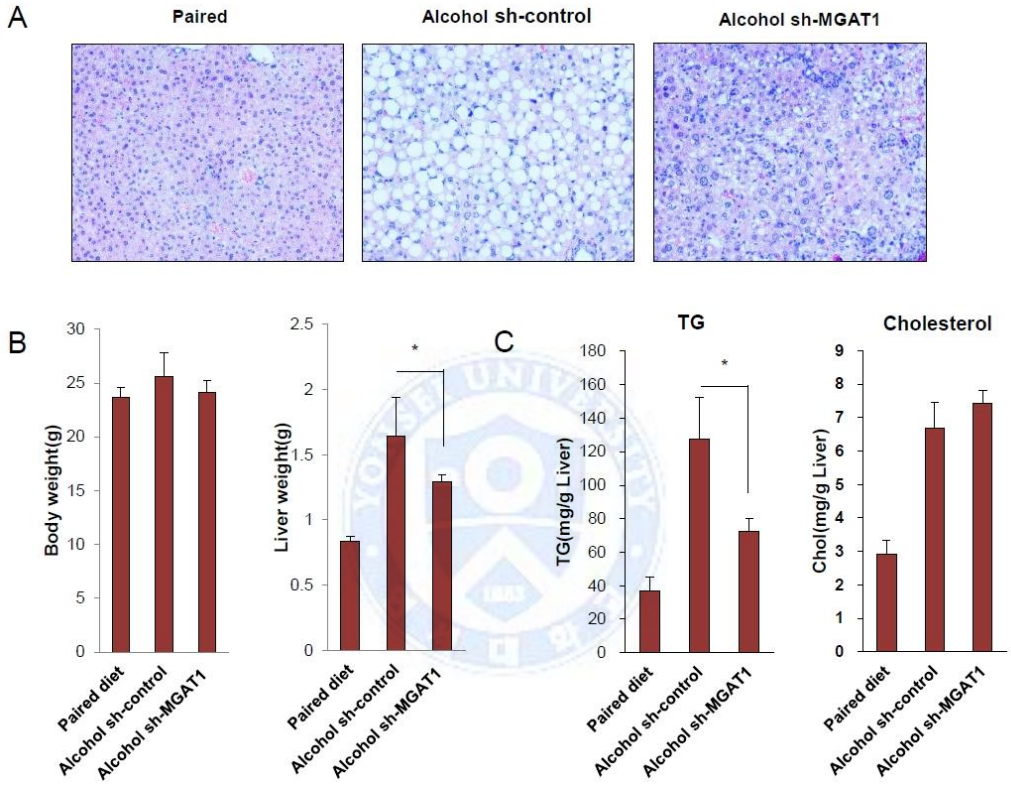


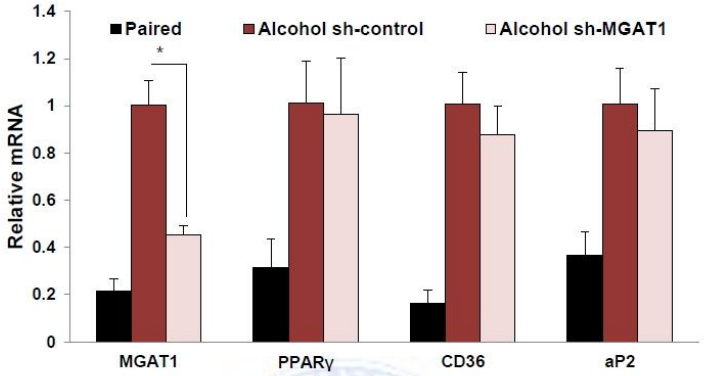

**Fig. 7. Ethanol metabolism in binge drinking.** (A) Schedule of binge drinking experiment. (B) $NAD^+$/NADH ratio checked in dexrin-maltose (DM) and ethanol-fed mice group. (n=6/group) (B) Western blot analysis showing the SIRT1 expression in DM and ethanol-fed mice group. (C, D) Real-time PCR analysis showing PPARγ, SREBP1c, ChREBP, and their target genes. n=6.

**E. MGAT1 knock-down protects against alcohol-induced hepatic steatosis**

To evaluate the effect of MGAT1 knock-down in alcohol induced hepatic steatosis, I performed sh-adenoviral transfection experiment. Male B6 mice were pair fed Liber-Decarli liquid diet with or without ethanol (27% of total calorie) for 3 wk and then were injected with adenoviral sh-control or sh-MGAT1 via tail vein. After 1 wk of continued Liber-Decarli liquid diet, mice were sacrificed for analysis. As shown in Fig. 8A, knockdown of hepatic MGAT1 significantly improved hepatic steatosis, without affecting the expression of PPARγ and its regulatory genes such as aP2/422 and CD36 (Fig. 8D). Hepatic TG level was decreased by 42% after only 1 week of MGAT1 knockdown (Fig. 8B). Furthermore, hepatic MGAT1 knockdown also resulted in decreased liver weight, suggesting that MGAT1 expression plays an important role in alcohol induced-hepatic steatosis (Fig. 8C). The biological parameters of these mice are shown in Fig. 5E, showing that plasma glucose level but not serum TG or cholesterol was changed by MGAT1 knockdown. It implies that MGAT1 knock-down can improve glucose metabolism.


A
Paired
Alcohol sh-control
Alcohol sh-MGAT1
B
Body weight(g)
Paired diet
Alcohol sh-control
Alcohol sh-MGAT1
C
Liver weight(g)
*
TG
TG(mg/g Liver)
*
Cholesterol
Chol(mg/g Liver)

D


Paired
Alcohol sh-control
Alcohol sh-MGAT1
Relative mRNA
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
MGAT1
PPARγ
CD36
aP2

E

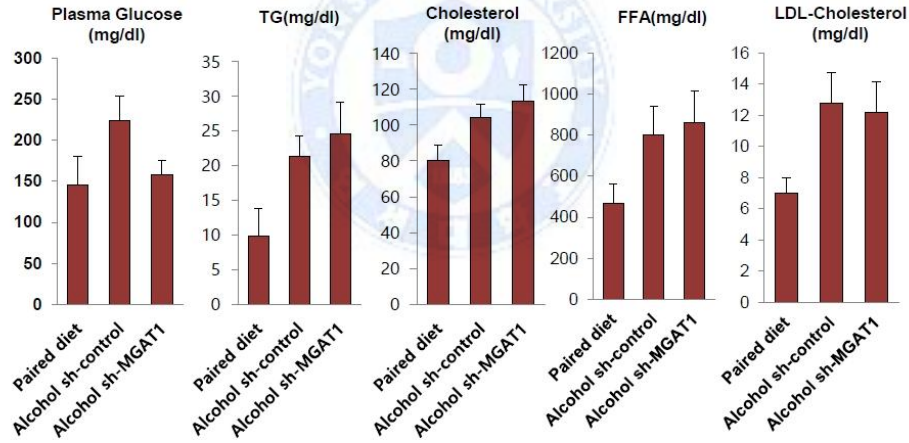

Plasma Glucose (mg/dl)
300
250
200
150
100
50
0
TG(mg/dl)
35
30
25
20
15
10
5
0
Cholesterol (mg/dl)
140
120
100
80
60
40
20
0
FFA(mg/dl)
1200
1000
800
600
400
200
0
LDL-Cholesterol (mg/dl)
16
14
12
10
8
6
4
2
0
Paired diet
Alcohol sh-control
Alcohol sh-MGAT1

**Fig. 8. MGAT1 knock-down suppresses alcohol-induced hepatic steatosis.**

(A) H&E staining performed on liver section in paired, EtOH sh-control, and EtOH sh-MGAT1 mice. (B) Body weight and liver weight. (C) Hepatic TG and cholesterol contents. (D) Real-time PCR analysis showing PPARγ, and its target genes. n=6. (E) Blood parameters after knock-down of MGAT1. Data in B, C, D and E represent the mean ± SD. *P < 0.05.

**F. Ethanol decreases $NAD^+$/NADH ratio and SIRT1 protein level in HepG2 cell, and SIRT1 suppresses PPARγ induced MGAT1 expression**

In HepG2 cell, the $NAD^+$/NADH ratio and SIRT1 level were decreased dose dependently by ethanol treatment (Fig. 9A, B). Next, to evaluate the effect of SIRT1 in PPARγ-induced MGAT1 expression, we proceeded with the Ad-PPARγ and Ad-SIRT1 transfection experiment using mouse primary hepatocyte. As shown Fig. 9C, SIRT1 efficiently suppressed the MGAT1 expression by PPARγ, and nicotinamide, known as a SIRT1 inhibitor, counteracted the suppressive effects of SIRT1. Luciferase assay also showed that SIRT1 suppresses PPARγ-induced MGAT1 promoter activity in HepG2 cell (Fig. 9D). To evaluate whether SREBP1c, another major transcription regulator of hepatic steatosis, regulates MGAT expression, I performed SREBP1c overexpression experiment in liver cell lines (Fig. 10). SREBP1c did not change the expression level of MGAT subtypes as PPARγ overexpression was done. Previously, PPARγ acetylation sites affected by SIRT1 were reported (at lysine residues 98, 107, 218, 268, and 293).[6] Therefore, we made PPARγ mutant forms (K98R, K107R, K218R, K268R, and K293R) to evaluate whether the acetylation sites, modulate PPARγ-induced MGAT1 expression. As shown in Fig. 11, we could not find a significant PPARγ acetylation site, which regulates MGAT1 expression. This result suggests that there may be other acetylation sites on PPARγ or that MGAT1 expression may be regulated by acetylation on several residues of PPARγ.

From the data shown here, I propose a model for the role of PPARγ and MGAT1 in alcohol-induced hepatic steatosis (Fig. 12). In the course of ethanol digestion, $NAD^+$ is converted into NADH by alcohol dehydrogenase and aldehyde dehydrogenase, and subsequently SIRT1 level decrease. Decreased SIRT1 activates SREBP1c, ChREBP and PPARγ. In early phase, SREBP1c and ChREBP's target genes were increased in expression, then PPARr target gene such as MGAT1, aP2, CD36 were increased. I think PPARr and its target gene expression has an important role in the development of an overt alcoholic steatosis.

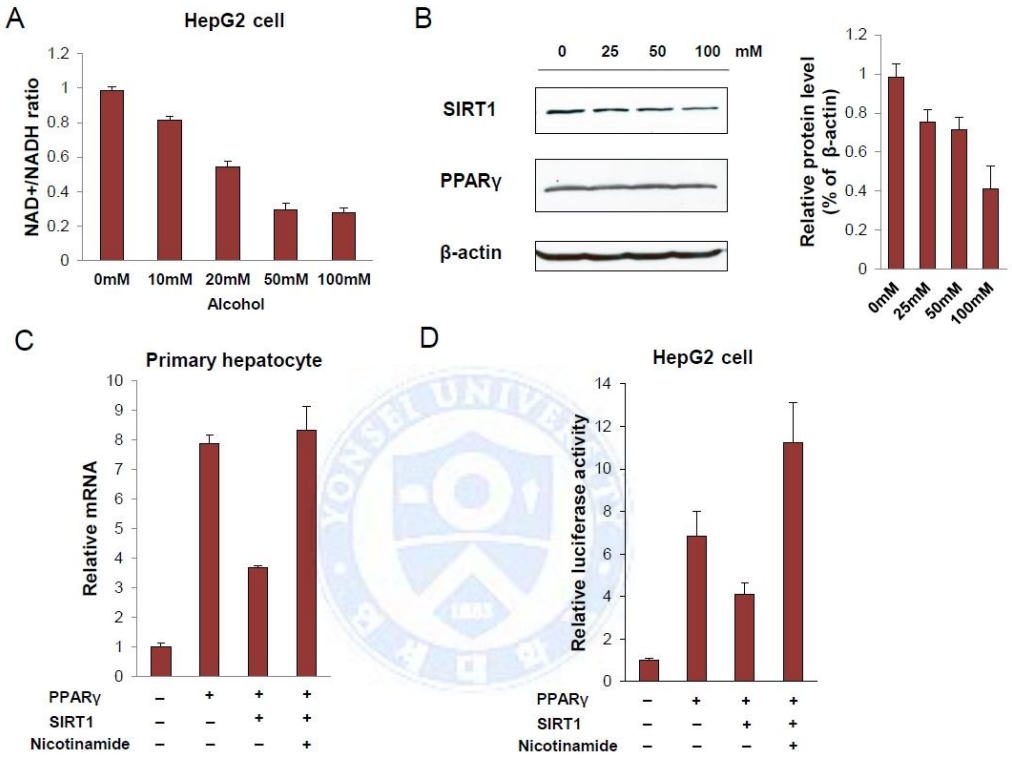

A
HepG2 cell
NAD+/NADH ratio
1.2
1
0.8
0.6
0.4
0.2
0
0mM
10mM
20mM
50mM
100mM
Alcohol
B
0
25
50
100
mM
SIRT1
PPARγ
β-actin
Relative protein level
(% of β-actin)
1.2
1
0.8
0.6
0.4
0.2
0
0mM
25mM
50mM
100mM
C
Primary hepatocyte
Relative mRNA
10
9
8
7
6
5
4
3
2
1
0
PPARγ – + + +
SIRT1 – – + +
Nicotinamide – – – +
D
HepG2 cell
Relative luciferase activity
14
12
10
8
6
4
2
0
PPARγ – + + +
SIRT1 – – + +
Nicotinamide – – – +

**Fig. 9. The effect of ethanol on $NAD^+$/NADH ratio and SIRT1 in liver cell, and MGAT1 regulation by PPARγ and SIRT1.** (A, B) Ethanol was treated at different concentrations to HepG2 cells. Next day, ethanol was treated one more time at the same doses, and cells were harvested after 24 hr. $NAD^+$/NADH ratio and western blot analysis were done. (C) Primary hepatocytes ($4X10^5$ per well) on 6- well plates were infected with 50MOI of Ad-GFP, Ad-PPARγ, and Ad-SIRT1. Nicotinamide (10mM). (D) Luciferase assay using human MGAT1 promoters (~ 2 kb) was performed. In HepG2 cells, MGAT1 promoter constructs were co-transfected with or without PPARγ/RXRα overexpression vectors using Lipofectamin 2000 reagent, and with 100 MOI of either Ad-GFP or Ad-SIRT1. The data were presented as mean ± SD from measurements collected from three independent experiments and two measurements for each experiment.

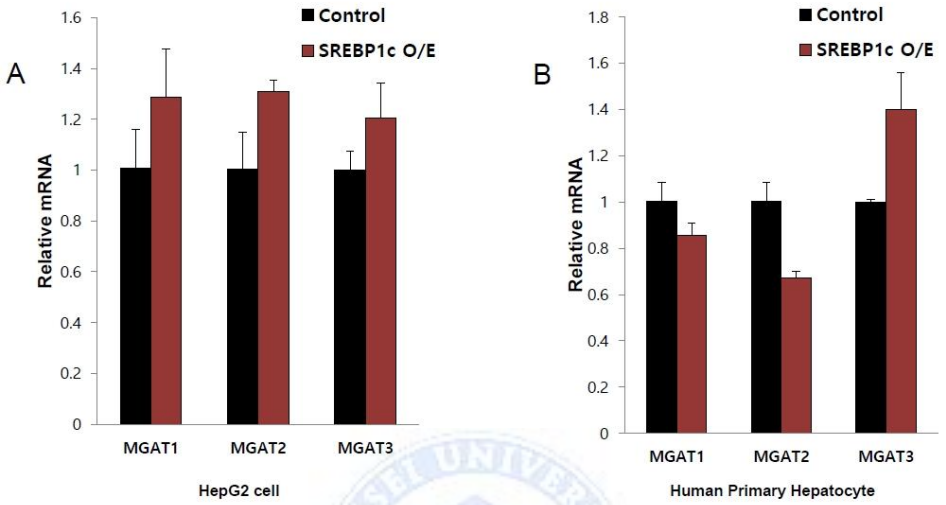



**Fig. 10. Expression patterns of MGATs to SREBP1c overexpression.** (A, B) HepG2 cells (4 x $10^5$ per well) on 6-well plates or human primary hepatocytes (1 x $10^6$ per well) were transfected with or without SREBP1c overexpression vectors using Lipofectamin 2000 reagent. After 24 hr, media change was done with 10% FBS-DMEM. After 48 hr, total RNA was prepared and analyzed by real-time qPCR. Data represent the mean ± SD.

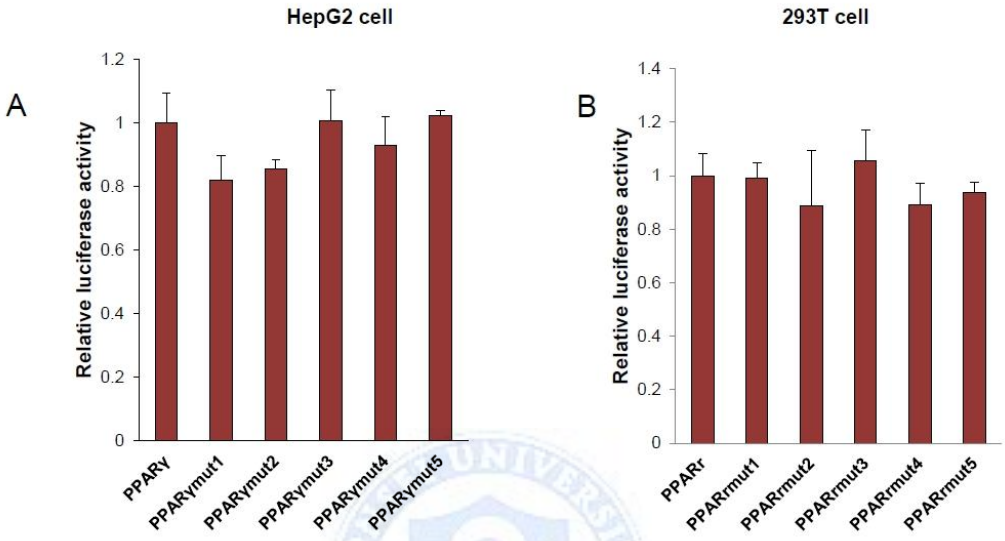



**Fig. 11. Luciferase assay to evaluate the acetylation sites, modulating PPARγ-induced MGAT1 expression.** Luciferase assay using mouse MGAT promoters (~ 2 kb of MGAT1 promoter) was performed. The promoter activity was shown by relative luciferase activity in (A) HepG2 cells or (B) 293T cells. MGAT promoter constructs were co-transfected with each PPARγ mutant forms using Lipofectamin 2000 reagent. The data were presented as mean ± SD from measurements collected from three independent experiments and two measurements for each experiment.

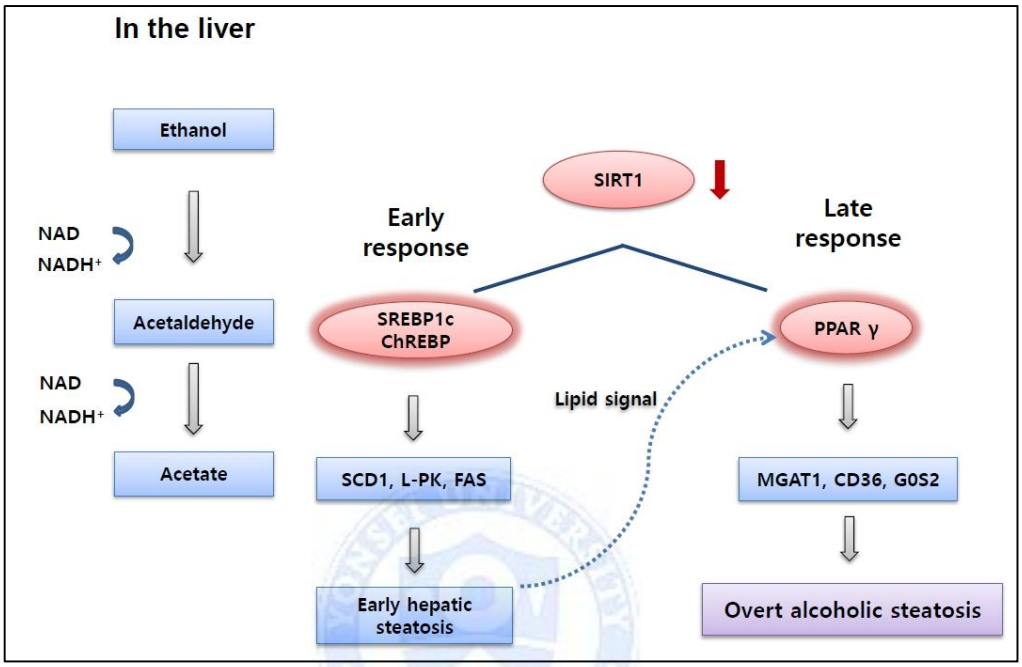



**Fig. 12. Model of alcohol-induced hepatic steatosis**. In the course of ethanol consumption, $NAD^+$ is converted into NADH by alcohol dehydrogenase and aldehyde dehydrogenase, and subsequently SIRT1 level decrease. SIRT1 activates SREBP1c, ChREBP and PPARγ. In the early phase, SREBP1c and ChREBP's target genes were increased in expression, and then PPARγ target genes such as MGAT1, aP2, CD36 were increased. PPARγ and its target gene expression lead to the development of overt alcoholic steatosis.

## 2. MGAT inhibitor development

### A. Screening method set up for MGAT activity check

To explore for MGAT inhibitor candidates, we set up new screening method using Ellman's reagent. It is very economical and efficient method compared to previous used CPM (7-Diethylamino-3-(4'-Maleimidylphenyl)-4-Methylcoumarin) method. Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB) is a chemical used to quantify the number or concentration of thiol groups in a sample.

We used monoacylglycerol and oleic-CoA as substrate, and MGAT enzyme purified from microsome of MGAT vector overexpressed 293T cell.
We performed the 1st screening using 2300 FDA-approved drugs. From these, 36 chemicals (Table 1) were shown to repress MGAT activity and based on the structures of these chemicals, 300 additional chemicals with similar structures were screened. The final candidates came down to 20 substances (Fig. 13). These 20 similarly-structured chemicals were shown to successfully repress MGAT activity both in CPM and Ellman's reagent (Fig. 14). To evaluate how these inhibitor suppresses MGAT subtypes, we have checked the inhibitive effect of MGAT subtypes. These drugs show similar repressive effect on MGAT2 as well (Fig. 15).

In vitro assay using HepG 2 cells, if we treat oleic acid and PPARr, we can make some lipid droplet in HepG2 cell. But, when we use our candidate chemical (B1758, 10 μM), I can barely find these lipid droplet formed (Fig. 16).

## MGAT inhibitor development progress

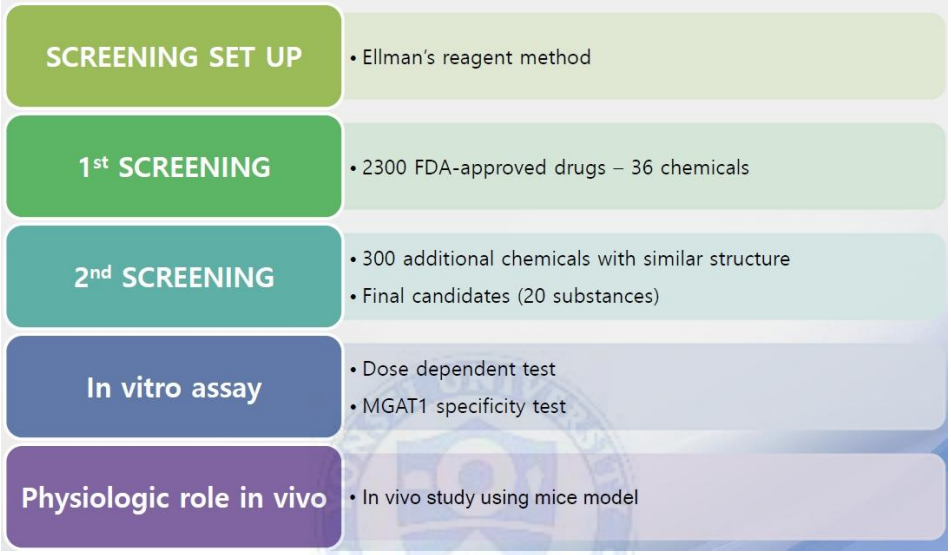



**Fig. 13. The progress in this screening procedure.** The 1st screening using 2300 FDA-approved drugs was done. From these, 36 chemicals were shown to repress MGAT activity and based on the structures of these chemicals, 300 additional chemicals with similar structures were screened. The final candidates came down to 20 substances.

**Table 1. 36 chemicals selected from 1st screening**

| Molecule | Inhibition(%) | Molecule | Inhibition(%) | Molecule | inhibition(%) |
|---|---|---|---|---|---|
| LIOTHYRONINE | 75.2 | NIFEDIPINE | 100.0 | CANDESARTAN CILEXTIL | 59.3 |
| DESOXYCORTICOSTERONE ACETATE | 83.8 | ERYTHROSINE SODIUM | 100.0 | PHENYLMERCURIC ACETATE | 100.0 |
| BITHIONATE SODIUM | 70.7 | NISOLDIPINE | 76.0 | CISPLATIN | 100.0 |
| CAPREOMYCIN SULFATE | 55.1 | METHYLENE BLUE | 97.5 | THIRAM | 63.8 |
| GLYBURIDE | 84.6 | QUINESTROL | 100.0 | MERBROMIN | 100.0 |
| CLOTRIMAZOLE | 65.9 | ECONAZOLE NITRATE | 60.8 | EBSELEN | 100.0 |
| DANAZOL | 50.8 | DALBERGIONE, 4-METHOXY-4'-HYDROXY- | 85.0 | ACRIFLAVINIUM HYDROCHLORIDE | 94.1 |
| HEXACHLOROPHENE | 81.4 | DALBERGIONE | 100.0 | BRONOPOL | 100.0 |
| MICONAZOLE NITRATE | 65.4 | AVERMECTIN A1a | 61.2 | EPIGALLOCATECHIN-3-MONOGALLATE | 65.8 |
| PROGESTERONE | 69.6 | CHLORANIL | 100.0 | EPIGALLOCATECHIN | 53.5 |
| THIOTHIXENE | 50.6 | TETRAHYDROGAMBOGIC ACID | 59.8 | ACONITIC ACID | 58.4 |
| FLUORESCEIN | 89.5 | 4-NONYLPHENOL | 79.1 | HAEMATOXYLIN | 72.5 |

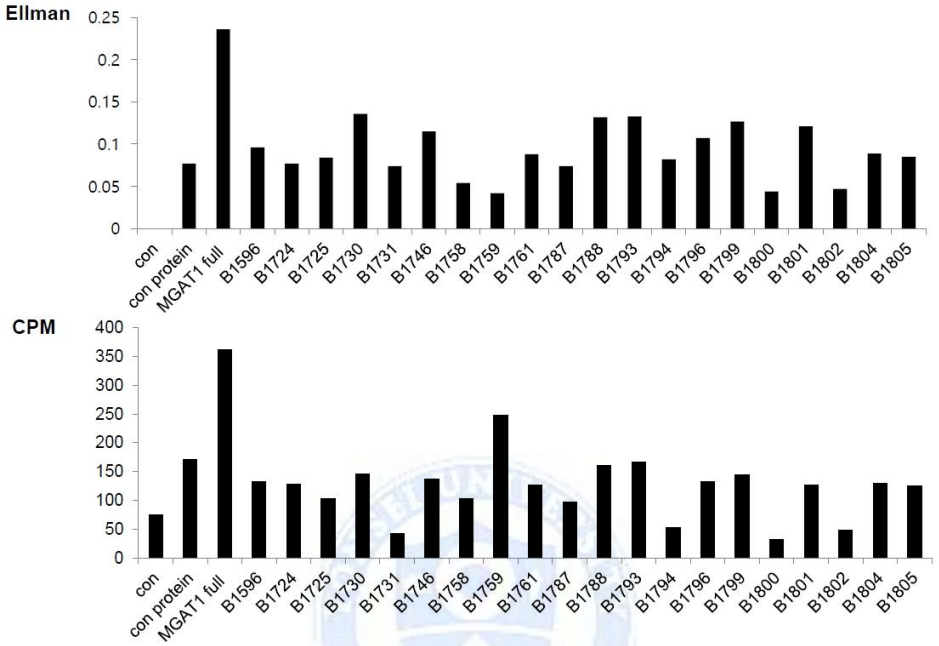



**Fig. 14. Final canadidate chemicals suppresses MGAT activity.** 20 similarly-structured chemicals show to successfully repress MGAT activity both in CPM and Ellman's reagent.

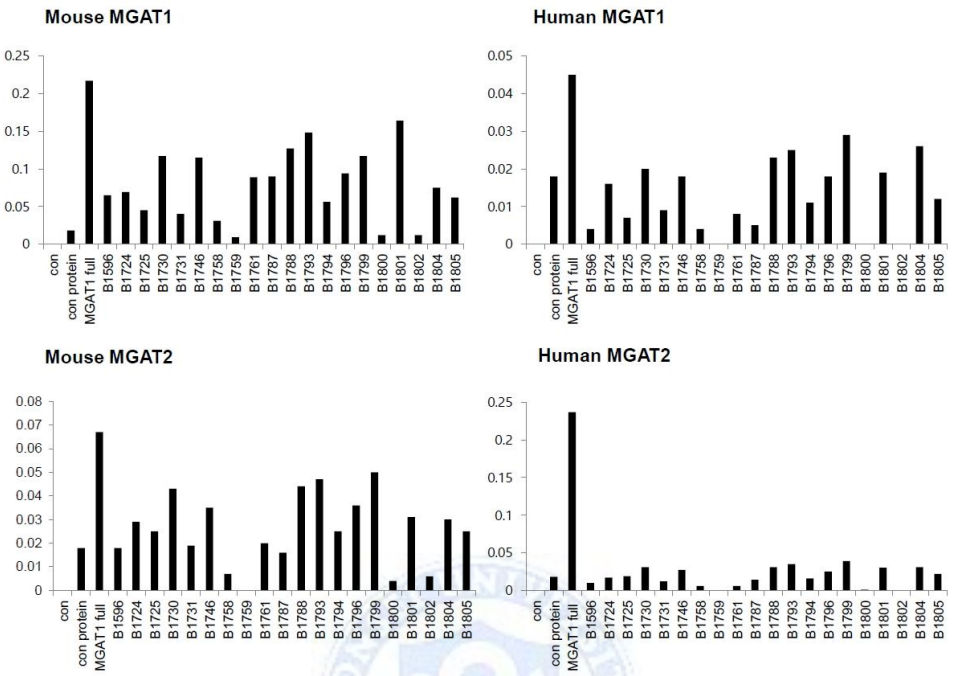



**Fig. 15. Inhibitor candidates suppresses MGAT subtypes.** The inhibitory effect of MGAT subtypes, these drugs show similar repressive effect on MGAT2 as well as MGAT1.

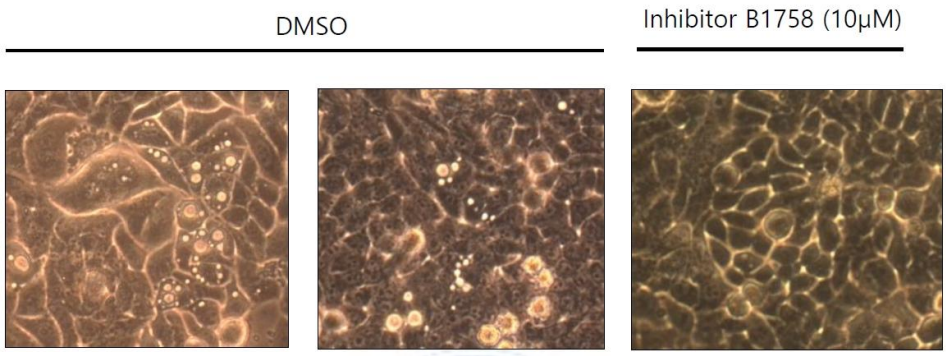



**Fig. 16. B1758 suppresses lipid accumulation in HepG2 cell.** HepG2 cell (2x $10^5$ per well) on 6-well plates were infected with 100 MOI of Ad-PPARγ. After 24 hr, MGAT inhibitor (B1758, 10μM) was treated with 40 μM oleic acid and 10% FBS-DMEM. After 48 hr, media change was done with 40 μM oleic acid and 10% FBS-DMEM. Pictures were taken 4 days after inhibitor treatment. B1758 successfully suppresses lipid formation in HepG 2 cell.

**B. In vivo effect of MGAT inhibitor (B1758)**

To study in vivo effect of MGAT inhibitor, we used ob/ob mice which are widely used in metabolic disease research. Male ob/ob mice were pair fed chow diet with or without inhibitor (B1758, 10 mg/kg) for 2 wk. Exposure to MGAT inhibitor for 2 wk resulted in a significant decrease in hepatic TG contents and liver weight, which means MGAT inhibitor successfully suppressed hepatic steatosis (Fig. 16B, C). Furthermore, MGAT inhibitor slightly attenuated adipocyte size (Fig. 17A) and body weight (Fig. 16B). H&E staining performed on liver section also shows the inhibitory effect of MGAT inhibitor on hepatic lipid accumulation (Fig. 17B).

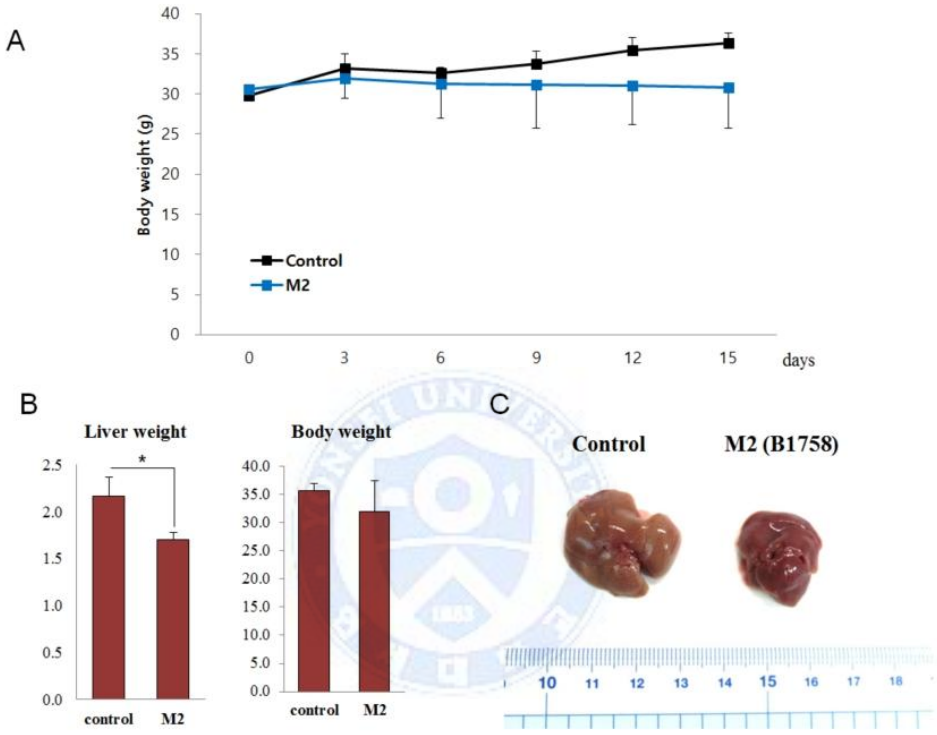



**Fig. 17.MGAT inhibitor decreases body weight and liver weight.** (A) Body weight for 2 wk of experiment. Data shows slight decreased body weight in inhibitor treated ob/ob mice group. n=3 (B) Live weight and body weight in each mice group. (C) Decreased liver mass in MGAT inhibitor treated group.

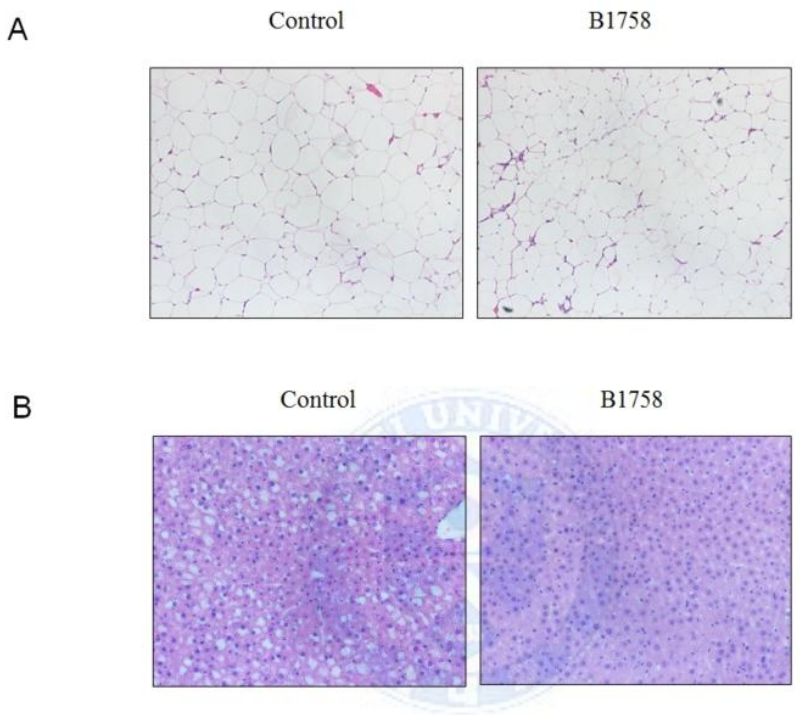



**Fig. 18. Histologic analysis in MGAT inhibitor treated ob/ob mouse liver.**

(A, B) H&E staining performed on white adipose tissue (epididymal fat) and liver section in control and MGAT inhibitor treated mice.

## IV. DISCUSSION

Excessive alcohol consumption is an important public health problem and contributes markedly to global burden of mortality and morbidity. In the 2010 Global Burden of Disease (GDB) study, alcohol-attributable liver disease was responsible for 493,300 deaths (156,900 females and 336,400 males) representing 0.9% of all global deaths.[19] Therefore, the efforts to reduce alcohol consumption and prevent the alcohol-induced hepatic damage are demanded. Alcoholic liver disease starts with hepatic steatosis, characterized by an increase in intrahepatic triacylglycerol, and continuous alcohol consumption leads to hepatic inflammation and liver fibrosis. It has been estimated that a third of patients with steatosis will develop hepatic inflammation and 8 to 20 % of patients with steatosis will eventually progress to cirrhosis.[1,20] The main causes for alcoholic fatty liver are known to be acetaldehyde, TNF-α, ER stress, 2-AG, and adenosine, which increase SREBP1c activity and induces fatty acid synthesis and alcoholic fatty liver. PPARα, known to be another major regulator, decreases fatty acid β-oxidation.[1] Recently, ChREBP's role during the early period to alcohol consumption was reported.[13] In case of PPARγ, it is known as a critical regulator for TG accumulation in adipose tissue. The role of PPARγ in alcohol-induced hepatic steatosis has not been investigated until now.

PPARγ is a ligand-activated transcription factor as a member of the nuclear receptor superfamily, mainly regulating adipogenesis and lipid accumulation.[21] Because of lower PPARγ expression in liver compared to that in adipose tissue, there are conflicting opinions on the role of PPARγ in the liver.[22] In the past decade, it was found that hepatic PPARγ is significantly increased in an obese animal model and has an important role in fatty liver formation.[8,23,24] In our study, alcohol-fed mice showed an increase in hepatic PPARγ protein level, to a certain extent, but not so evidently, when compared to control mice. However, the target genes of PPARγ are increased in alcohol-fed mice. Therefore, we investigated the post-translational modification of PPARγ to examine how ethanol regulates PPARγ activity.

Until now, post-translational modifications known to regulate PPARγ function are phosphorylation, acetylation, sumoylation and ubiqutination.[25] PPARγ is phosphorylated within AF1 region by mitogen-activated protein kinases (MAPKs) or cyclin-dependent kinases (Cdk7 and Cdk9), which conflictingly regulate PPARγ activity.[26,27] Moreover, it was found that PPARγ is phosphorylated within the LBD at Ser273 by Cdk5 that regulates the expression of a distinct group of genes that are aberrant in obesity.[28] Sumoylation of PPARγ in the AF1 region represses its transcriptional activity, possibly by recruiting co-

repressor.[29] Additionally, PPARγ has been shown to be ubiquitinated, which is enhanced by ligand binding like TZDs.[30,31] It is not elucidated how PPARγ function is regulated by acetylation. However, some studies have reported that PPARγ activity is associated with acetylation.[32-34] Especially, PPARγ activity is attenuated by deacetylase such as HDAC3 and SIRT1.[5,35]

NAD-dependent deacetylase 1 called sirtuin 1 (SIRT1) regulates lipid metabolism by deacetylating lysine residues on transcription factor, such as SREBP1c and PGC1α in liver.[36-38] However, it was not investigated how SIRT1 regulates PPARγ function, which is known as an important transcription regulator in fat mobilization, especially in alcoholic fatty liver. In this study, I have established that SIRT1 is involved in alcoholic hepatic steatosis by regulating the acetylation of PPARγ and its activity. Furthermore, I can see that PPARγ overexpression via adenovirus tail vein injection could induce hepatic steatosis. Therefore, I consider PPARγ signaling pathway is very critical in hepatic lipid synthesis and fatty liver formation. Furthermore, MGAT1 knock-down, PPARγ target gene, could inhibit hepatic lipid accumulation. MGAT1 enzyme is associated only with monoacylglycerol pathway, the inhibition of MGAT activity does not completely block TG synthesis. On the other hand, diacylglycerol acyltransferase (DGAT) inhibition can definitely repress TG

synthesis. Therefore, DGAT inhibitor development was done vigorously in other studies, but side effects still prevent the development its clinical model. In obesity mice model, the expression of enzymes involved in glycerol phosphate pathway was not as much induced as for MGAT1 and the inhibition of MGAT1 expression efficiently decreased the formation of hepatic steatosis. In conclusion, I think that developing MGAT1 inhibitor would effectively counter hepatic steatosis without side effects.

Over the past few decades, we categorize the cause of hepatic steatosis, alcoholic or non-alcoholic; however it is hard to determine clinically which type it is. Furthermore, alcohol synergistically increases the prevalence and the severity of hapatic steatosis in obese patients.[39,40] Discovering similar pathway between non-alcoholic hepatic steatosis and alcoholic hepatic steatosis is very useful strategy of finding therapeutic target of hepatic steatosis. Here, I identified that the SIRT1-PPARγ-MGAT1 axis is involved in alcohic hepatic steatosis. We previously reported the important role of PPARγ and MGAT1 in non–alcoholic hepatic steatosis.[8] Threrefore, I suggest that the study of PPARγ and its target gene such as MGAT1 could be a potential therapeutic target of hepatic fatty liver, which is increasing with obesity and metabolic disease.

## VI. CONCLUSION

1. Ethanol reduces Sirt1 expression and its activity, thereby inducing PPARγ acetyl-ation and increases PPARγ target gene expressions.
2. PPARγ and its target genes have an important role during hepatic lipid accumulation.
3. MGAT1 knock-down efficiently attenuated alcohol-induced hepatic steatosis.
4. MGAT1 is a potential therapeutic target of alcoholic or non-alcoholic hepatic steatosis, so MGAT1 inhibitor development would be an effective method to treat hepatic steatosis.

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Abstract (IN KOREAN)

# MGAT1 유전자의 알코올성 지방간에서의 역할 규명 및 MGAT1 억제를 통한 지방간의 치료

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유정환

알코올은 지방간의 중요한 원인으로 지속적으로 알코올을 섭취할 경우 간 섬유화, 간경변 및 간암을 일으킨다. 지방간은 당뇨와 같은 대사질환과 연관되어 있으며 간 질환 발생의 출발선상에 존재한다. 하지만 지금까지 알코올이 어떻게 지방간을

형성하는지 그 기전은 명확하지 않다. 본 연구를 통해 알코올성 지방간 형성에 $NAD^+$/NADH ratio, sirtuin1 (SIRT1) 및 peroxisome proliferator-activated receptor γ (PPARγ) 가 어떻게 작용하는지 알아보고자 하였다. 알코올이 대사되는 과정 중에 $NAD^+$ 는 alcohol dehydrogenase 과 aldehyde dehydrogenase 에 의해 NADH 로 변환되고, 다음 단계로 $NAD^+$에 의존하는 SIRT1 의 발현이 감소되게 된다. SIRT1 의 감소는 지방 세포의 분화 및 지방 형성에 중요한 PPARγ 의 acetylation 을 증가시키게 되고, 증가된 acetylation 은 PPARγ 의 활성을 증가시킨다. 활성화 된 PPARγ 는 지방 형성에 중요하다고 알려진 MGAT1 유전자를 증가시키고 알코올성 지방간 형성을 촉진시킨다. 더불어 MGAT1 유전자의 발현 감소는 알코올성 지방간 형성을 현격하게 감소시킨다. 이상의 결과로 PPARγ 와 MGAT1 이 비알코올성 지방간 형성뿐만 아니라, 알코올성 지방간 형성에 중요하다는 사실을 알 수 있었다.

다음으로 MGAT 억제제를 개발하기 위해 MGAT 활성을 측정할 수 있는 Ellman 용액을 이용한 검사 방법을 확립하고, FAD 승인을 받은 2300 여개의 약물을 가지고 MGAT 효소 활성 억제를 측정하였다. 측정 결과 MGAT 활성 억제 효과를 보이는 약물 36 여개를 찾을 수 있었고 추가적으로 농도 별 활성 측정을 통해 보다 효과가 좋은 약물을

추려내었다. 이후 이들 약물과 구조가 비슷한 화학물질을 찾아 MGAT 효소 활성 억제 효과가 있는 약물을 찾아 내었고, 특정 구조를 가진 약 20 개의 후보 물질을 찾아 낼 수 있었다. 이러한 후보물질은 동물 실험에서 효과적으로 지방간을 억제 시키는 결과를 확인할 수 있었다. 결과적으로 이러한 MGAT1 억제제의 개발은 치료하지 않으면 간경변과 같은 만성 간질환으로 진행할 수 있는 알코올성 지방간 및 비알코올성 지방간 치료에 큰 도움이 될 것으로 생각한다.

핵심되는 말 : $NAD^+$/NADH ratio, SIRT1, PPARγ, MGAT1, alcoholic steatosis

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