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석사학위 논문

Establishment of *in vitro* model and therapeutic target identification for the hepatic lipotoxicity

조선대학교 대학원

약 학 과

김 재 훈

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지방독성 간세포 모델 확립 및 이를 활용한

치료표적 발굴 연구

2020 년 2 월 25 일

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이 논문을 약학 석사학위신청 논문으로 제출함

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ABBREVIATIONS

ActD	Actinomycin-D
ACC	Acetyl-CoA carboxylase
BSA	Bovine serum albumin
DMSO	Dimethylsulfoxide
DCFH-DA	2’,7’-Dichlorofluorescein diacetate
EtOH	Ethanol
FAS	Fatty acid synthase
HCD	High cholesterol diet
HFD	High fat diet
H_2O_2	Hydrogen peroxide
mTOR	Mammalian target of rapamycin
mTORC1	Mammalian target of rapamycin complex 1
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
PA	Palmitic acid
ROS	Reactive oxygen species
REDD1	Regulated in development and DNA damage response 1
SREBP-1	Sterol regulatory element-binding protein 1
TG	Triglyceride

국 문 초 록

지방독성 간세포 모델 확립 및 이를 활용한 치료표적 발굴 연구

김 재 훈

지 도 교 수: 기 성 환

약학과

조선대학교 대학원

지방독성은 과도한 지질 축적으로 인해 비지방조직에 지질이 축적되어 세포의 기능장애 및 사멸로 이어지는 대사 증후군의 대표적인 원인으로 알려져 있다. 우리 몸에 들어온 지질은 저장을 위해 지질 중간체로 전환될 수 있는데 전환된 지질 중간체들은 일차적으로 지방세포에 축적이 된다. 지방세포가 포화 상태가 되면 쌓인 지질들이 비지방세포에 영향을 미치게 되고 결국 지방독성을 일으키게 된다. 지질이 과다 축적된 비지방세포는 지방독성으로 인해 혈장 내 유리 지방산 수치를 상승시켜 인슐린 민감성에 부정적인 영향을 끼치고, 근육 내 지질 중간체의 축적으로 미토콘드리아의 기능장애를 유발하며, 간 손상을 초래한다. 간세포에서의 과량의 지질 축적은 산화적 스트레스를 유발하여 활성산소종을 생성하고 세포 사멸과 염증 그리고 비알콜성 지방간과 같은 간 질환을 초래한다. 하지만 이러한 간 지방독

성 연구에 적합한 세포 (*in vitro*) 모델이 확립되어 있지 않기 때문에 본 연구에서는 여러 간 세포주에 지방독성을 유도하는 것으로 알려진 Palmitic acid 를 처리하여 지방독성 연구에 가장 적합한 세포를 구축하고자 하였다. 또한 지방간을 유발하는 에탄올 식이 모델과 고콜레스테롤 식이, 고지질 식이 모델의 간 조직에서 REDD1 단백질의 발현이 증가하는 것을 확인하였다. REDD1 은 활성산소종, 세포 내 에너지 결핍, DNA 손상 등 다양한 스트레스에 반응하여 세포 생존에 관여하는 단백질로 알려져 있다. 그러나 간세포에서 과량의 지질 축적에 의해 유발되는 지방독성에 대한 REDD1 의 역할과 조절 기전에 대해서는 알려진 바가 없다. 본 연구에서 지방독성을 유도하는 Palmitic acid 를 처리하였을 때 REDD1 의 발현이 증가하는 것을 확인하였다. 또한 Palmitic acid 에 의한 REDD1 발현의 증가는 전사적 활성화에 의해 조절되었다. 더 나아가 REDD1 을 세포 내에 과발현 시켰을 때 지방독성을 유도하는 Palmitic acid 로 인한 세포사멸을 방어하여 세포 생존율을 증가시키며 지질 축적을 감소시켰다. 이러한 결과를 종합해 보았을 때 REDD1 이 간 지방독성 및 이로 인한 간질환의 새로운 치료 표적이 될 수 있을것이다.

I. INTRODUCTION

Fatty liver is a condition where lipid accumulation in more than 5% of hepatocytes. Non-alcoholic fatty liver disease (NAFLD), one of the most common liver diseases in the world, is caused by various factors. The most closely related factors of NAFLD are obesity and metabolic syndrome and the accumulation of triglycerides (TG) in hepatocytes is hallmark of NAFLD [1]. Fatty liver, whether alcoholic fatty liver disease or NAFLD, can progress to late-stage liver diseases ranging from cirrhosis to liver cancer [2].

Lipotoxicity is major cause of metabolic syndrome in which lipid intermediates accumulate in non-adipose tissues leading to dysfunction and cell death [3]. In normal cellular tissue, there is a balance between lipid production, oxidation and transport. But in lipotoxic-induced cells, there is an imbalance between the amount of lipid produced and the amount of lipid used [4]. These lipids can be converted to lipid intermediates such as triacylglycerol, diacylglycerol, ceramides, and Fatty acyl-CoAs for storage in cells, where lipids accumulate in adipocytes that serve as reservoirs and accumulate in non-adipocytes when adipocytes become saturated [5]. Lipids that accumulate in non-adipocyte eventually raise plasma levels of free fatty acid, negatively affecting insulin sensitivity [6], and also causing mitochondrial dysfunction by the accumulation of Fatty acyl-CoA in muscle [7]. As a result, non-adipocyte with excessive lipid accumulation results in cell dysfunction or death due to lipotoxicity, and further lead to a diversity of diseases such as liver fibrosis, NAFLD, type 2 diabetes and obesity [8].

The protein Regulated in DNA Damage and Development 1 (REDD1; also referred to as Rtp801 and DDIT4) has been shown in several studies to act as an inhibitor of mTORC1 signaling [9]. The mTORC1 orchestrates cell growth in response to several environmental inputs such as growth factor and nutrient availability, as well as hypoxia and energy stress [10]. In addition, REDD1 was identified as a hypoxia inducible factor 1 target gene involved in the

regulation of cell survival [11]. Additional studies reported regulation of REDD1 in response to other cellular stresses including hypoxia and glucocorticoid treatment [12]. Thus, it is well established that REDD1 is related to cell survival in response to various cell stress. However, the role of REDD1 in hepatocellular injury by excessive fatty acid accumulation has not been investigated.

In this study, we investigated the effects of Palmitic acid (PA) on HepG2, H4IIE, and Huh7 cells, in order to present an *in vitro* model for hepatic lipotoxicity. Also, we observed cell viability, ROS production, lipid accumulation, and lipid metabolism-related proteins in each cell, suggesting that Huh7 cells are most sensitive against hepatic lipotoxicity. In addition, the expression of REDD1 was increased when Huh7 cells were treated with PA, and overexpression of REDD1 affected cell survival and lipid accumulation in hepatocytes. In addition, it was confirmed that the expression of REDD1 is increased in hepatic tissue from high-fat diet animal. Conclusively, we demonstrated here that REDD1 might be a promising therapeutic target for the lipotoxicity and its related liver diseases.

II. MATERIALS AND METHODS

1. Materials

Antibodies against Sterol regulatory element-binding protein 1 (SREBP-1) were provided by Santa Cruz Biotechnology (Santa Cruz, CA). Regulated in development and DNA damage response 1 (REDD1) antibody was obtained from Proteinteck (Chicago, IL). Acetyl-CoA carboxylase (ACC), Fatty acid synthase (FAS) antibody was obtained from Cell Signaling Technology (Danvers, MA, USA). Horseradish peroxidase-conjugated anti-rabbit and anti-mouse antibodies. 2', 7'-dichlorofluorscein diacetate (DCFH-DA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), Dimethylsulfoxide (DMSO), bovine serum albumin (BSA), Palmitic acid (PA), Oil Red O, Actinomycin-D (ActD), and β-actin antibody were purchased from Sigma Chemicals (St. Louis, MO).

2. Cell culture

HepG2, H4IIE and Huh7 cell lines were purchased from ATCC (American Type Culture Collection, Manassas, VA). HepG2 , H4IIE cells were maintained in DMEM containing 10% fetal bovine serum, 50 units/ml penicillin/ streptomycin and Huh7 cell were maintained in RPMI-1640 containing 10% FBS, 50 units/ml penicillin/ streptomycin at 37°C in a humidified 5% CO_2 atmosphere

3. Animal and diet

Animal studies were conducted under the guidelines of the Institutional Animal Use and Care Committee at Chosun University, Gwangju, South Korea. C57BL6 mice were obtained from Oriental Bio (Sungnam, Korea) and acclimatized for 1 week. We obtained liver tissue from three animal models to evaluate the expression of REDD1 as previously reported [13, 14, 15]. Mice were sacrificed, and tissue samples were collected. REDD1 protein levels were measured

in the liver homogenates.

4. PA preparation

To prepare a PA stock solution, dissolve 10 mM PA in phosphate buffered saline without BSA for 5 h at 70°C. The molar ratio is PA and BSA, 2:1. This PA stock solution is dissolved in MEM medium by the addition of BSA when processed. At this time, the ratio of BSA is 2%. The vehicle group is treated after dissolving only BSA except PA. All Fatty acids treatments were performed with a 0.45 μm syringe filter before treatment.

5. MTT assay

The measure cytotoxicity, HepG2, H4IIE, Huh7 cells were plated at a density of 1 x 10^5 cells/well in 12 well plates and incubated with Palmitic acid (1 mM) for 24 h. After treatment, viable cells were stained with MTT (0.2 mg/mL and incubated for 1 h). The media were then removed and any formazan crystals produced in the wells were dissolved with the addition of 300 μl of dimethyl sulfoxide. Absorbance at 550 nm was measured using an enzyme-linked immunosorbent assay microplate reader (Spectra MAX, Molecular Device, Sunnyvale, CA). Cell viability was defined relative to the vehicle [i.e., viability (% vehicle) = 100 x (absorbance of treated sample) / (absorbance of control)].

6. RNA isolation and RT-PCR analysis

Total RNA was extracted using Trizol (Invitrogen, Carlsbad. CA) according to the manufacturer's instructions. To obtain cDNA, total RNA (1 μg) was reverse-transcribed using an oligo (dT) $_{16}$ primer. The cDNA was amplified using a high capacity cDNA synthesis kit (Bioneer, Daejeon, Korea). PCR was conducted using a PCR premix (Bioneer Daejeon, Korea) with a thermal cycler (Bio-Rad, Hercules, CA). Primers were synthesized by Bioneer. The following primer sequences were used: human REDD1 Sense 5'-

GAGCCTGGAGAGCTCGGACT-3' and antisense 5'-CTGCATCAGGTTGGCACACA-3'; human Glyceraldehyde-3-phosphate dehydrogenase(GAPDH) sense 5'-GAAGGTGAAGGTCGGAGTC-3' and antisense 5'-GAAGATGGTGATGGGATTTC-3'. GAPDH was used as a reference gene for normalization.

7. Plasmid transfection and luciferase assay

The human REDD1 promoter driven luciferase reporter construct, pcmv-REDD1, was kindly provided by RIKEN BRC through the National Bio resource Project of MEXT, Japan. To determine the activities of the REDD1 promoter constructs, we used the dual-luciferase reporter assay system (Promega).

8. Immunoblot analysis

Cell lysates were prepared according to the previously published methods [16]. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and immunoblot analyses were performed according to previously published procedures [16]. Briefly, the cell lysates were separated using 7.5%, 6% gel electrophoresis, The protein were then electophoretically transferred to nitrocellulose membranes. After the membranes were blocked, they were incubated with primary antibody at 4℃ overnight and then incubated with a horseradish peroxidase-conjugated secondary antibody (Zymed Laboratories, San Francisco, CA). The immune reactive protein was visualized using an ECL chemiluminesence detection kit (Amersham Biosciences, Buckinghamshire, UK). β-actin was used as immunoblotting controls. Three separate experiments were performed with different lysates to confirm the changes in the protein levels.

9. Preparation of recombinant adenovirus.

To generate adenoviral REDD1 construct, full-length human REDD1 open reading frame was amplified by using attB-fused specific primers, subcloned to pDONR221 (Invitrogen), and

inserted into pAD/CMV/V5- DEST gateway plasmid (Invitrogen). Recombinant adenovirus was produced from HEK293A cells, according to the manufacturer's instruction (Invitrogen). The DNA sequences of the constructs were verified using ABI 3730 XL DNA sequencer (Solgent, Daejeon, Korea). Transduction of recombinant adenovirus was conducted, as previously described [16]. Adenovirus which expresses LacZ (Ad-LacZ) was used as an infection control.

10. Oil red O staining

Lipid accumulation was observed by Oil red O staining. To measure the lipid accumulation by Oil red O staining, after removing media, it was washed with phosphate buffered saline 500 μl (12 well standard). Add 500 μl of 10% formalin per well and incubate at room temperature for 1 h. After that, it was washed several times with distilled water and let it dry completely. Add 500 μl of 0.7% Oil red O (Sigma-aldrich Co., USA) solution and stain at room temperature for 1 h. After that, remove Oil red O solution, wells were added 500 μl of 85% propylene glycol (Junsei Chemical Co., Japan) for 5 min, and wash 5 times with distilled water. Finally, add 300 μl of isopropyl and incubate for 20 min at room temperature. Absorbance at 450 nm was measured using an enzyme-linked immunosorbent assay microplate reader.

11. Measurement of ROS production

DCFH-DA is a cell-permeable, non-fluorescent probe that is cleaved by intracellular esterases and is converted into a highly fluorescent dichlorofluorescein when it reacts with H_2O_2. After treatment, the HepG2, H4IIE and Huh7 cells were stained with 10 mM DCFH-DA for 30 min at 37℃. H_2O_2 generation was determined by dichlorofluorescein fluorescence. Fluorescence intensity in the cells was measured using a fluorescence microplate reader (Gemini, Molecular Devices, Sunnyvale, California) at excitation/emission wavelengths of 485 nm/525 nm.

12. statistical analysis

One-way analysis of variance (ANOVA) was used to determine the significances of differences between treatment groups. The Newman-Keuls test was used to determine the significances of differences between multiple group means. Results are expressed as means ± SE.

III. RESULTS

1. Cytotoxicity induced by PA in hepatocytes

First, we investigated the cytotoxic effect of PA in hepatocytes. Cell viability was dose dependently reduced in Huh7 cells, but not in H4IIE cells. In case of HepG2, cell viability was significantly reduced in 2 mM PA treatment only (Fig. 1A). It is well known that reactive oxygen species (ROS) levels was increased by PA treatment [17]. Therefore, we confirmed ROS production after PA treatment using fluorescence microplate reader. In cells ROS levels were peaked at 0.5 mM PA treatment for 24 h (Fig. 1B). These results suggest that excessive fatty acid treatment has a cytotoxic effect in hepatocytes.

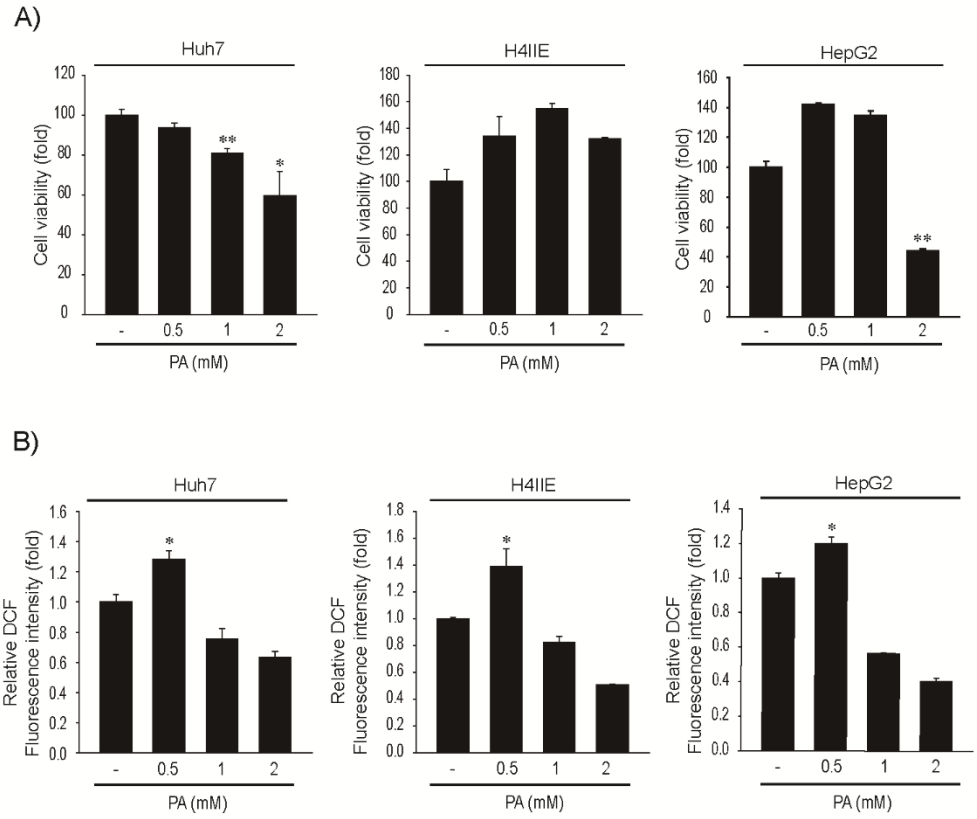


Figure 1. The cytotoxic effect of PA in hepatocytes.

(A) The effect of PA on cytotoxicity. Cells were exposed to PA (0.5 mM, 1 mM, 2 mM) for 24 h. Cell viabilities were assessed using an MTT assay. (B) The effect of PA on ROS production. Cells were stained with 1 μM DCFH-DA for 30 min at 37℃. Intracellular fluorescence intensities were measured using a flow-cytometry analysis. Data represent the mean ± S.E. of three separate experiments; the statistical significance of differences between each treatment group and the control (**$p<0.01$, *$p<0.05$).

2. Lipid accumulation by PA in hepatocytes.

Lipid accumulation by PA was investigated using Oil red O staning (Fig. 2A). Lipid accumulation was significantly increased by treatment with 1 mM PA in hepatocytes. Quantification of Oil red O levels exhibited a substantial increase in lipid accumulation, which was in line with the results of microscopy images (Fig. 2B). Huh7 cells were found to increase the expression of ACC, FAS, SREBP-1 protein at 6, 12 h after PA treatment. However, PA increased FAS expression but did not increase ACC and SREBP-1 expression in H4IIE. In HepG2 cells, ACC and FAS were slightly increased, but SREBP-1 expression was not changed. Based on these results, Huh7 cells are considered to be an *in vitro* model for hepatic lipotoxicity studies.

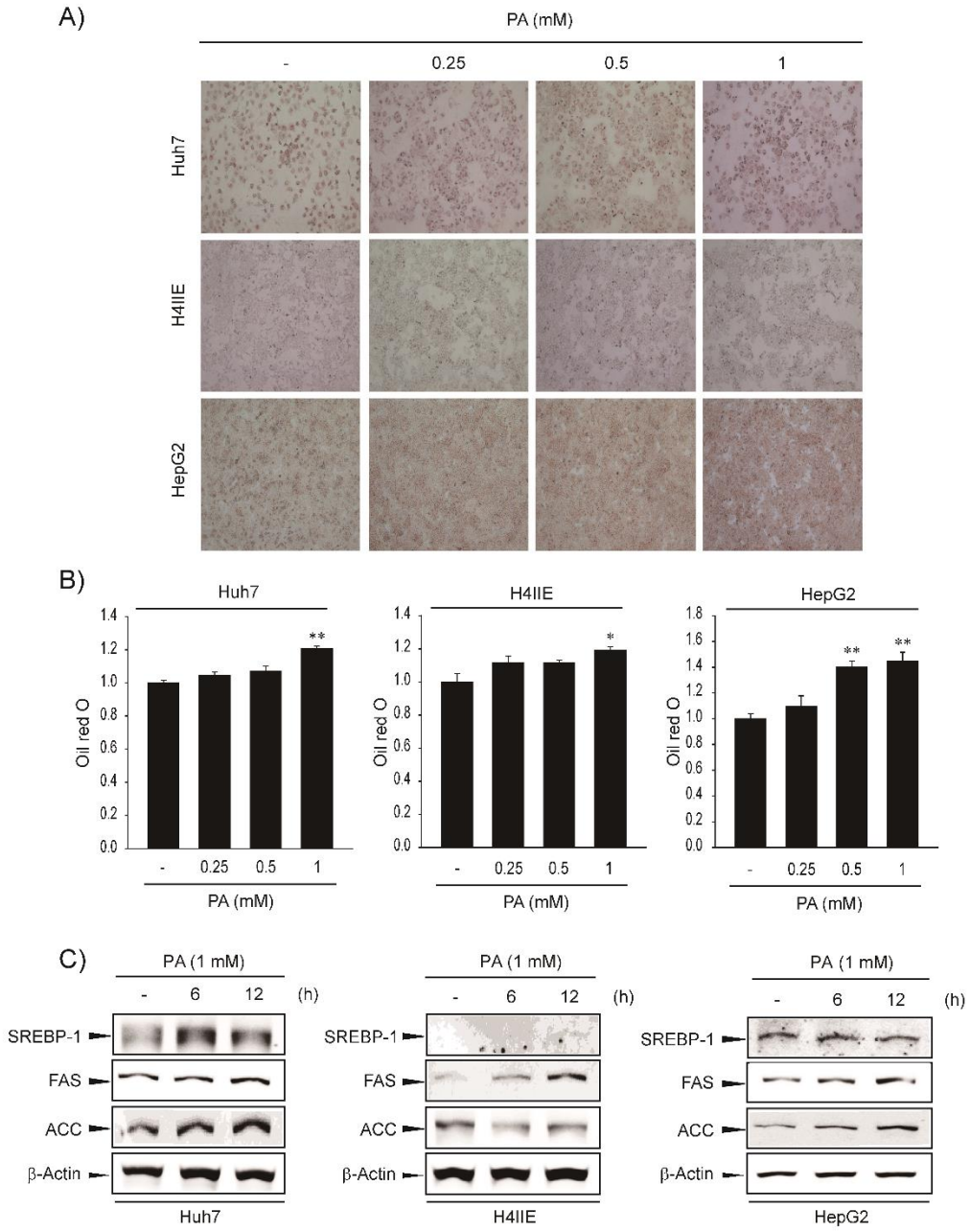


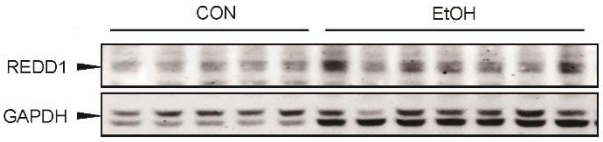
Figure 2. The effect of PA on lipid accumulation in hepatocytes.

Hepatocytes were exposed to PA (0.25 mM, 0.5 mM, 1 mM) for 24 h. (A) Cells were stained with Oil red O. (B) Lipid levels of Oil red O stained cells were quantified, respectively. Data represent means ± S.E. of three replicates; significant as compared with vehicle treated control cells, *$p<0.05$, **$p<0.01$. (C) Immunoblot analyses for lipid metabolism-related proteins were carried out in hepatocytes treated with PA (1 mM) for 6, 12 h, respectively.

3. Expression of REDD1 in *in vivo* fatty liver model.

Chronic Ethanol (EtOH) feeding, High cholesterol diet (HCD), High fat diet (HFD) is widely used as an *in vivo* model for studying the fatty liver disease [18, 19]. We investigated the expression of REDD1 in several liver disease *in vivo* models. First, the expression level of REDD1 protein in the chronic EtOH model was examined by immunoblotting. The expression level of REDD1 protein was increased in the alchol-containing (EtOH) diet group compared to the normal diet group (Fig. 3A). Next, the expression level of REDD1 in the HCD model was examined by immunoblotting. The expression level of REDD1 was increased in the HCD group compared to the control diet group (Fig. 3B). In addition, the expression levels of REDD1 was increased in the HFD group compared to the control diet group (Fig. 3C). These results show that REDD1 expression is increased in fatty liver *in vivo* model.

A)



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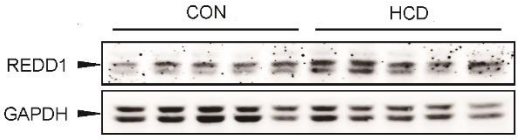

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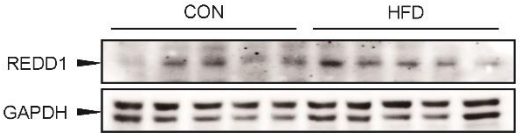


Figure 3. Induction of REDD1 in fatty liver animal model.

(A) Mice were fed with pair-fed control or alcohol-containing diet (EtOH) for 4 weeks. REDD1 protein was immunoblotted in samples prepared from mice treated alcohol-containing diet. (B) The mice were fed either a normal diet or HCD for 10 weeks. REDD1 protein was immunoblotted in samples prepared from mice fed HCD. (C) The mice were fed either a normal diet or HFD for 8 weeks. REDD1 protein was immunoblotted in samples prepared from mice fed HFD.

4. Increased expression level and transcriptional regulation of REDD1 by PA.

REDD1 is known to be regulated in response to cell survival, DNA damage, nutrient depletion, glucocorticoids and insulin [11]. But, the mechanism of REDD1 gene expression in stress condition due to excessive lipid accumulation is unveiled. So, we examined the time course of REDD1 expression in response to treatment with 1 mM PA. REDD1 protein level was increased after 6–24 h of PA treatment, which was peaked at 12 h (Fig. 4A). We investigated using RT-PCR analysis whether the induction of REDD1 by PA is due to increased transcription. The results showed that PA was increased the level of REDD1 mRNA (Fig. 4B). Moreover, the luciferase activity of human REDD1 promoter was measured by treated cells with PA (Fig. 4C). Time-dependent treatment of PA (1 mM) in Huh7 cells increased REDD1 luciferase activity. When Huh7 cells were co-treated with the transcription inhibitor Act-D adding PA (1 mM). Act-D treatment completely inhibited increase in REDD1 levels induced by PA (Fig. 4D).

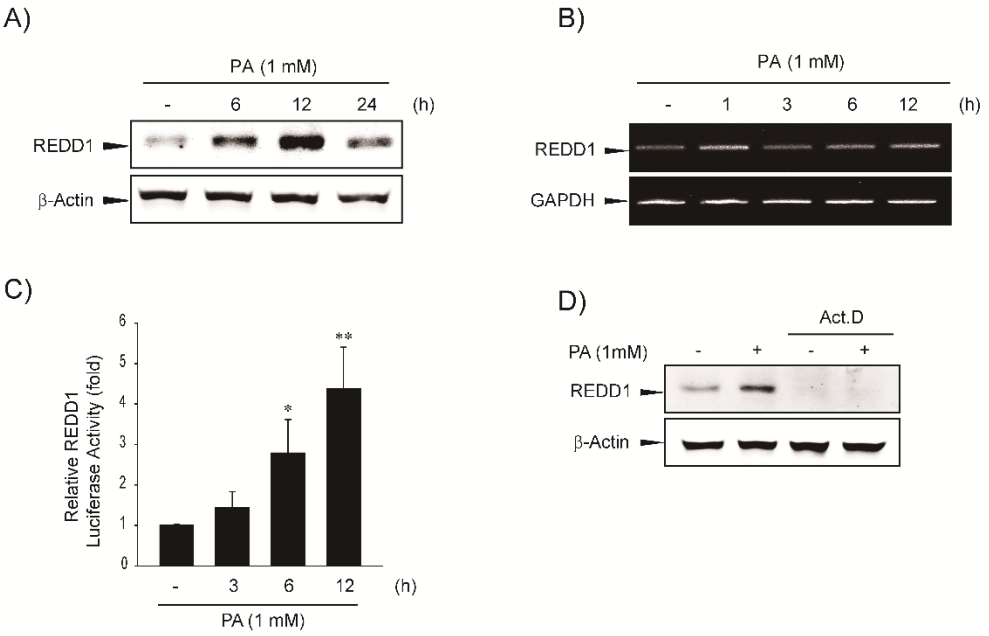
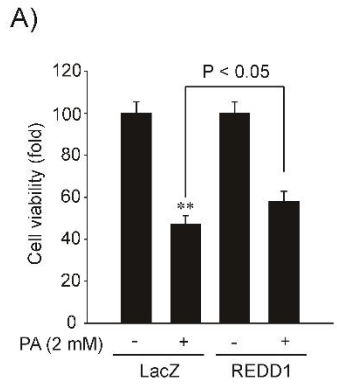


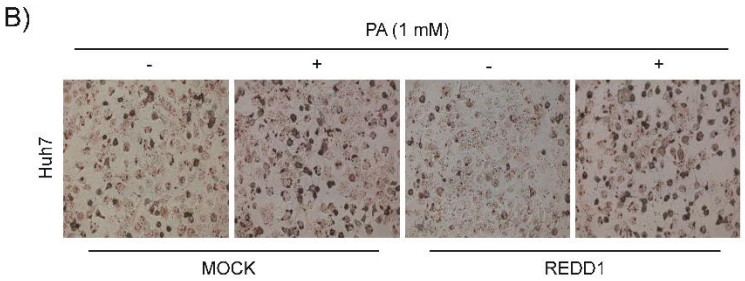
Figure 4. Induction of REDD1 by PA in hepatocytes.

(A) Huh7 cells were exposed to PA (1 mM) for 6 h, 12 h, 24 h. REDD1 protein levels in lysates were measured by immunoblotting. (B) RT-PCR analysis. REDD1 transcript levels were determined after cells were treated with PA (1 mM) for 3-24 h. (C) REDD1-luciferase activity was determined in the lysates of Huh7 cells treated with PA (1mM) for 3 h, 6 h, 12 h. Data represent means ± S.E. of three replicates; significant as compared with vehicle treated control cells, *$p<0.05$, **$p<0.01$ (D) The effect of Actinomycin-D (ActD) on REDD1 transcriptional regulation by PA (1mM) in Huh7 cells. The cells were treated with ActD (5 μg/mL) in presence or absence of PA (1 mM) for 6 h.

5. The cytoprotective effet of REDD1 against lipotoxicity.

We investigated the functional role of REDD1 on PA-induced cell death and lipid accumulation. First, we measured cell viability using MTT assay in ad-LacZ or ad-REDD1 transfected Huh7 cells after treated PA. The decreased cell viability by PA was moderately elevated by REDD1 overexpression (Fig. 5A). Next, we performed Oil red O staining in REDD1 overexpressed Huh7 cells to determine the effect of REDD1 on lipid accumulation (Fig. 5B). Lipid accumulation was slightly decreased by REDD1 overexpression. Also, quantification of Oil red O levels exhibited decrease in lipid accumulation by REDD1 overexpression, which was in line with the results of microscopy images (Fig 5C). Our results indicate that REDD1 inhibits PA cytotoxicity and lipid accumulation in hepatocytes.





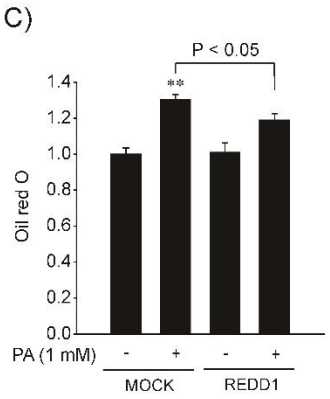


Figure 5. Preventive effect of REDD1 against PA-induced lipotoxicity.

(A) Huh7 cells transduced Ad-REDD1 or ad-LacZ for 3 h, treated with PA (2 mM) for 30 h, and measured cell viability by MTT assay. (B) Cells were stained with Oil red O. (C) Oil red O stained cells were quantified. Data represent means ± S.E. of three replicates; significant as compared with vehicle treated control cells, **$p<0.01$.

IV. DISCUSSION

Hepatic lipotoxicity is known as a cause of NAFLD previous [4]. Hepatic lipotoxicity affects insulin resistance and mitochondrial ROS production [20]. It was also known to induce ER stress, cell death [21], and apoptosis and autophagy [22]. Lipotoxicity is a cause of several diseases, including the heart, liver, kidneys, and skeletal muscle [7]. Lipotoxicity in the liver occurs due to excessive accumulation of triglycerides in hepatocytes, which impairs cell function and especially disrupts cell homeostasis. Hepatic lipotoxicity leads to oxidative stress, lipid peroxidation, and cell death, leading to liver fibrosis or NAFLD [5]. However, it is a lack of research on the therapeutic target and therapeutics for hepatic lipotoxicity. The reason is that there are no good animal and cell models for lipotoxicity studies.

We investigated to establish *in vitro* model for hepatic lipotoxicity studies. We conducted the study in several hepatocytes, such as Huh7, HepG2, and H4IIE. PA is known as free fatty acids that causes lipotoxicity [23]. We treated hepatocytes with PA and examined cytotoxicity, ROS production levels, lipid accumulation and expression of lipid-related proteins. PA treatment in Huh7 cells reduced cell viability in a concentration dependent manner. However, PA treatment in H4IIE and HepG2 cells did not decrease cell viability in a concentration dependent manner (Fig. 1A). PA increased ROS levels (Fig. 1B) and lipid accumulation in all used hepatocytes (Fig. 2B). ACC, FAS and SREBP-1, expression levels were known to be important proteins for lipid formation and accumulation in hepatocytes [24]. In H4IIE cells, expression of FAS was increased, but ACC was decreased, and SREBP-1 was not changed. HepG2 cells increased expression of ACC and FAS but decreased expression of SREBP-1. However, Huh7 cells were found to increase the expression of both ACC, FAS, SREBP-1 (Fig. 2C). As a result, it was confirmed that Huh7 cells are the most reliable cells for the study of hepatic lipotoxicity considering the cytotoxicity, ROS production level, lipid accumulation level and expression level of lipid-related protein by PA.

REDD1 is a protein known to be regulated by DNA damage, energy stress, hypoxia and various cellular stresses [10, 11, 12, 25]. REDD1 is known to inhibit mTOR signaling, and REDD1-mediated inhibition of mTOR is stimulated in cells exposed to hypoxia and in cells subjected to energy stress and relies on the presence of a functional TSC1 / TSC2 tumor suppressor complex [26]. Lipotoxicity causes a variety of stress situations, including cellular stress, energy imbalance, and ROS production following lipid accumulation. Therefore, we speculated that REDD1 might affect hepatic lipotoxicity.

First, we observed an increase in REDD1 protein expression in fatty liver animal models including alcohol, high cholesterol and high fat diet (Fig. 3). However, the role and function of REDD1 in hepatic lipotoxicity has not been studied yet. We investigated REDD1 in Huh7 cells based the results of increased expression levels of REDD1 protein in fatty liver animal models. In addition, we found that time-dependent increase in protein expression levels of REDD1 was achieved by treatment of PA in Huh7 (Fig. 4A). Induction of REDD1 by PA was transcriptionally regulated as evidence luciferase assay and mRNA (Fig. 4C). Furthermore, we examined the effect of REDD1 on lipotoxicity by PA. After overexpression of REDD1 in Huh7 cells, PA was treated to investigate cell viability and lipid accumulation. When REDD1 overexpressed, it was increased cytotoxicity by PA was reduced (Fig. 5A). Similarly, lipid accumulation by PA was reduced by overexpression of REDD1 (Fig 5C). However, further studies are still required to ensure that these results are caused by REDD1. In addition, REDD1 effect against lipotoxicity should be investigated in animal model.

In conclusion, the results of this study showed that Huh7 cells are appropriate cell model for lipotoxicity study, and suggest that REDD1 is a promising therapeutic target for hepatic lipotoxicity and NAFLD.

V. REFERENCES

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