의학석사 학위논문

Study on role of PHF2 in the development of non-alcoholic fatty liver disease.

지방간 발달과정에서 PHF2 의 역할에 관한 연구

2016 년 2 월

서울대학교 대학원

의학과 의과학전공

송 정 엽

A thesis of the Master's Degree

지방간 발달과정에서 PHF2 의 역할에 관한 연구

Study on role of PHF2 in the development of non-alcoholic fatty liver disease

February 2016

The Department of Biomedical Sciences,
Seoul National University
College of Medicine
Jung-Yup Song

Study on role of PHF2 in the development of non-alcoholic fatty liver disease.

by

Jung Yup Song

A thesis submitted to the Department of Biomedical Sciences in partial fulfillment of the requirements for the Degree of Master of Science in Medicine at Seoul National University College of Medicine

December 2015

Approved by Thesis Committee:

Professor Kong-Wan Park Chairman
Professor Yang-Sook Chun Vice chairman
Professor Ju-Hong Jeon

지방간 발달과정에서 PHF2 의 역할에 관한 연구

지도교수 전 양 숙

이 논문을 의학석사 학위논문으로 제출함
2015 년 12 월

서울대학교 대학원
의과학과 의과학전공
송 정 엽

송정엽의 의학석사 학위논문을 인준함
2015 년 12 월

위 원 장 박 종 완 (인)

부위원장 전 양 숙 (인)

위 원 전 규 홍 (인)

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is caused by excessive fat accumulation in the hepatocytes. Mild steatosis may develop into aggressive forms of Hepatic fibrosis, cirrhosis and carcinoma. Plant homeodomain finger 2 (PHF2), a JmjC histone demethylase, is known as the demethylase of the Histone H3K9 while it binds to H3K4me3 with the PHD domain. PHF2 is known to have associated with metabolism-related transcription factors. In this study, we identified the role of PHF2 in the progression of hepatic steatosis.

For in vivo study, WT and PHF2 overexpressed TG mice were fed normal diet or high fat diet for 8 weeks. And then, Liver steatosis and blood chemistry were analyzed. The expression level of protein and genes involved in liver lipid metabolism were measured. For in-vitro study, PHF2 was stably silenced in HepG2, human liver cell line.

Overexpression of PHF2 attenuated lipid accumulation in liver and insulin tolerance in mice fed with high fat diet. The expression of lipogenic genes was decreased in TG mice liver. In in vitro study, increased levels of lipogenic genes were

confirmed in PHF2 knock-down stable cell. In addition, we found that PHF2 directly interacts with SREBP1c through protein-protein interaction.

These results suggest that PHF2 plays a role as a repressor in the progression of hepatic steatosis through the association with SREBP1.

Keywords: Non-alcoholic fatty liver diseas;, Hepatic steatosis; Plant Hoemeodomain finger2; PHF2; SREBP1c;

Student number: 2013-23529

CONTENTS

LIST OF TABLES AND FIGURES

LIST OF ABBREVIATION

NAFLD: Non-alcoholic fatty liver disease

SREBP1c: Sterol regulatory element binding protein-1c

PHF2: Plant homeodomain finger protein 2

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is continuously rising globally as an important risk factor of liver disease. The first fibrotic progression of NAFLD is non-alcoholic hepatitis (NASH) that may further progress into fibrosis or cirrhosis, which could develop into hepatocellular carcinoma. (Marchesini, 2003) About 9 to 36% of the population in different countries suffers from NAFLD and the prevalence is increasing. (Ekstedt, 2006 & Lazo, 2011) Underlying mechanism of NAFLD is unclear and needs to be more elucidated. Over nutrition, repartitioning of TG from white adipose tissue, de novo synthesis and insulin resistance are known as risk factors of NAFLD. (Lee, 2014& Donnelly, 2005)

Imbalance regulation between lipid uptake and disposal brings about lipid accumulation in hepatocytes. (Fan, 2013) Several transcriptional factors and nuclear receptors are reported to be critical for the hepatic lipid homeostasis. (Lien, 2013) Sterol regulatory element binding protein-1c (SREBP1c) is identified as one of the most prominent lipogenic regulators that exists as a precursor form in ER membrane, but transforms to an active

form following the post-translational modification. (Fortz, 1999 & Sakai, 1998) SREBP1c activates the expression of approximately 30 genes that are involved in fatty acid synthesis and uptake. (Ikarshi, 2011) Under normal condition, insulin stimulates SREBP1c that enhances transcription for lipogenesis, such as fatty acid synthase (FAS) and acetyl-coenzyme A carboxylase (ACC). (Brown and Goldstesin, 1997) However, it has been reported that SREBP1 pathway is maintained in insulin resistant condition. (Shimomura, 2000) Therefore, active SREBP1 levels are increased, and triglycerides accumulation is accelerated.

Plant homeodomain finger protein 2 (PHF2) belongs to a family of Jumonji protein, carrying two domains, plant homeodomain (PHD) and a Jumonji-C domain at its N-terminus.(Fortschegger and Shiekhattar, 2011) JmjC domain removes the methyl groups from histone lysine residue, following the PHD domain binding to Lysine 4 tri-methylated histone 3 (H3K4me3). (Wen, 2010) Many studies report that PHF2 activates the expression of target genes by removing the repressive mark H3K9me2. Especially, for adipogenesis, it has been reported that PHF2 positively controls adipogenesis through interaction with CEBPα and

CEBP δ . (Lee, 2014) On the other hand, repressive role of PHF2 also has been reported for acting as a recruiter of H3K9me2/3 methyltransferase SUV39H1. (Guang, 2014) Thus, the exact role of PHF2 related histone demethylation needs to be more elucidated.

In this present study, I have investigated the role of PHF2 in liver lipid metabolism using PHF2 overexpressed mice and PHF2 knock down liver cell line. I found that PHF2 negatively regulates the lipid accumulation in liver through interaction with SREBP1c.

MATERIALS AND METHODS

1. Animal studies (blood chemistry, GTT, ITT)

C57BL/6 mice (Central Lab. Seoul, Korea) were housed under specific-pathogen free facility in a 12 h light/ 12 h dark cycle. 4 weeks old mice were fed with normal diet or high fat diet (60% fat of calories, Research Diets Inc., New Brunswick, NJ) for 12 weeks. Body weight and food consumption were measured once a week. Blood sample were collected using a capillary tube. Tissues were collected under anesthesia, weighed and divided into two parts for fixation and deep-frozen. For blood test, Serum was isolated from mice after fasting for 12 h. For glucose tolerance test, mice were fasted for 12 h and then injected D-glucose intraperitoneally at a dosed of 2 g/kg body weight. The plasma level of glucose was measured 0, 20, 40, 60, 90, and 120 mins after glucose injection using the glucometer Accu-Chek Active® system (Roche, Basel, Switzerland). Liver tissues were fixed with 4% formalin and stained with H&E solution.

2. Cell culture, gene silencing and treatment

HepG2 cell line was purchased from the Korean Cell Line Bank (Seoul, South Korea). The cell were cultured in Dulbecco's

Modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin and streptomycin in 5% CO_2 at 37 ℃. Oligonucleotides of control shRNA and PHF2 shRNA was inserted into pLKO.1 puro vector using AgeI and EcoRI restriction enzymes. The cells were infected overnight with virus. TO901317, an LxR agonist(Sigma-aldrich, 1 μg/ml) and an equal volume of DMSO (Control) were treated for 24 hours. The cell was incubated in media without FBS for 24 hours before the treatment.

3. Materials

Antibodies against SREBP1(sc-336), β-tubulin(sc-9104) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). PHF2 (3497S), FAS (3180S), H3K92me (9753S), H3K4me2 (9725S), and K3K27me2 (9728S) were purchased from Cell signaling (Danvers, MA, USA). Dulbecco's Modified Eagle's medium, fetal bovine serum (FBS) were purchased from Thermo Fisher Scinetific (Waltham, MA, USA). Trypsin, penicillin/streptomycin were purchased from Invitrogen (Carlsbad, CA, USA). Protein A/G-sepharose beads were purchased from GE Healthcare (Amersham, England). High Fat

Diet was purchased from Central Lab. Animal Inc. (Seoul, Korea). TO901317 was purchased from Sigma Aldrich.

4. RNA isolation and RT-qPCR

Total RNA was extracted using Trizol reagent (Invitrogen) according to the manufacturer's instructions. (Ambion, TX, USA) cDNA synthesis and amplification was performed using Multiscribe reverse transcription kit (Applied Biosystems, USA). Quantitative PCR was performed by EvaGreen 2X qPCR Master Mix-ROX (abm) with Stephone Real-Time PCR system (Applied Biosystems.). 18S ribosomal RNA was used as an internal control. Details of used primer sequences are listed in Supplementary table 1.

5. Western Blotting

Frozen Tissues were lysed in RIPA buffer (150 mM NaCl, 50 mM Tris-Cl, 0.5% NP-40, 1% Triton X-100) containing protease inhibitors. Lysates were separated on SDS-Polyacrylamide gels, and transferred to an Immobilon-P membrane (Millipore, USA). Membrane were blocked with 5% skim milk in TTBS for 1hour, and then incubated with a primary antibody (1:1000-1:5000)

overnight at 4 ℃. Membranes were incubated with the horseradish peroxidase-conjugated secondary antibody for 1 hour, and then developed using the ECL-plus kit (Thermo Scientific, Rockford, IL, USA).

RESULTS

PHF2 TG mice reduced diet-induced Hepatic steatosis.

In order to understand the role of PHF2 in the regulation of hepatic lipid metabolism, I first fed WT and PHF2 TG mice with normal diet or high fat diet. PHF2 TG mice were generated from previous study. (Kim, 2014) During the HFD feeding for 8 weeks, TG mice gained less body weight than the WT mice. (Fig 1A) I further performed the glucose tolerance test and insulin tolerance test to evaluate the insulin resistance. The TG mice fed with HFD showed significantly improved performances in both GTT and ITT. (Fig 1B) These results show that PHF2 may have an important role in the regulation of liver lipid homeostasis and NAFLD.

The morphology of livers appeared to be much red, which indicates less fat accumulation. (Fig 2A) Interestingly, HFD feeding resulted in large lipid droplets in WT mice liver, whereas TG liver formed smaller lipid droplets as shown by Hematoxylin and eosin staining. (Fig 2A) However, the weight of livers was not significantly different. (Fig2 B)

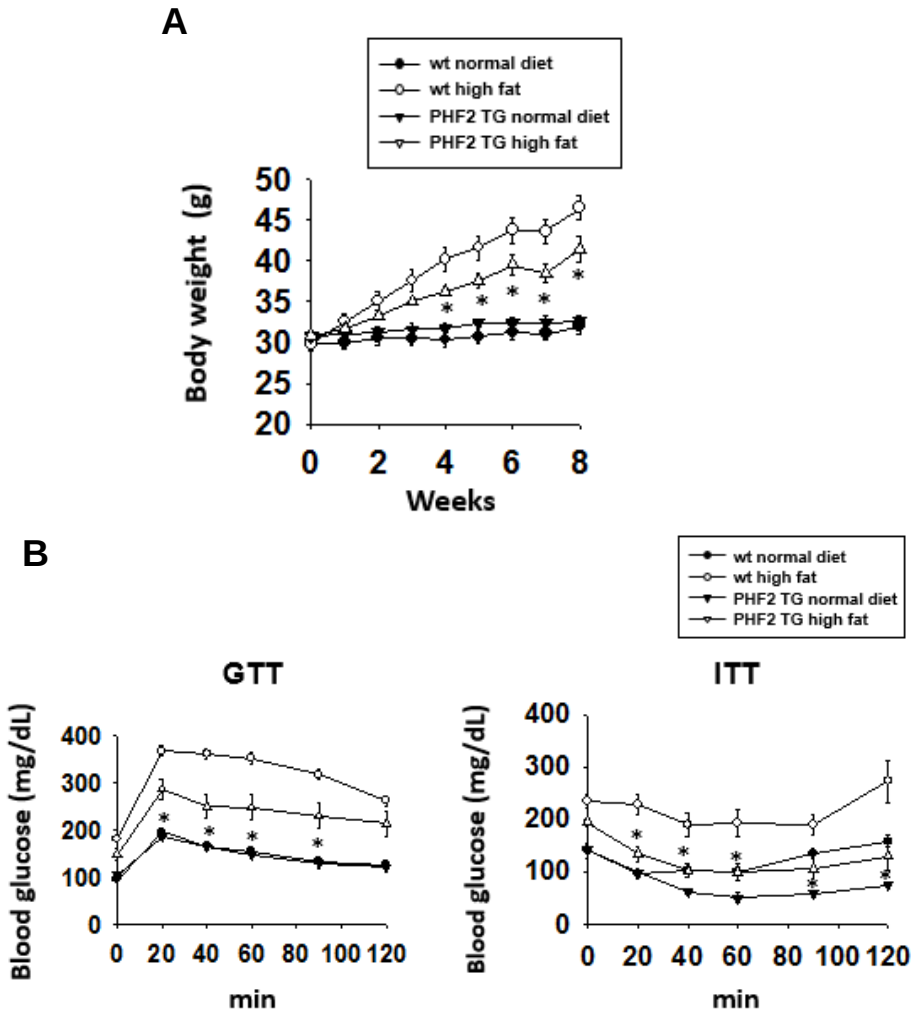
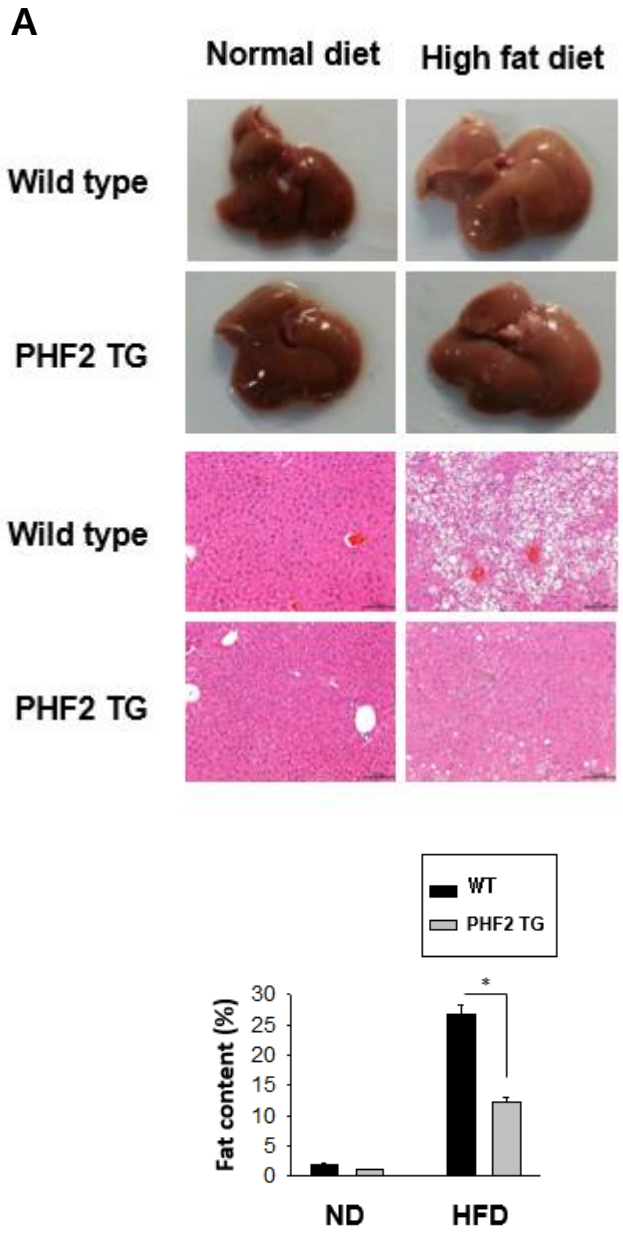


Figure 1. PHF2 TG mice fed with HFD gain less weight and insulin Resistance. (A)Body weight of each group was measured for 8 weeks. (B) GTT was performed after 12 h fasting and ITT was 6 h fasting. Compared to WT mice, TG mice displayed less glucose intolerance and insulin resistance. Data shown represent the mean ± SEM (n=8 mice/group) and * denotes $P<0.05$


A
Normal diet
High fat diet
Wild type
PHF2 TG
Wild type
PHF2 TG
WT
PHF2 TG
Fat content (%)
30
25
20
15
10
5
0
*
ND
HFD

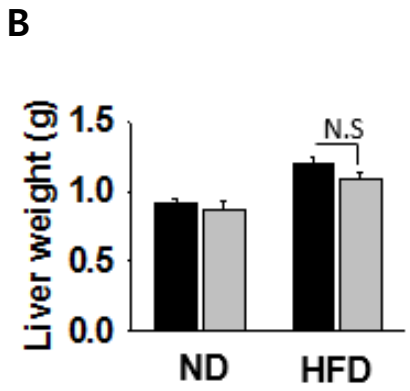


Figure 2. Hepatic steatosis is attenuated in HFD-TG mice. (A) Represent pictures of WT and TG liver fed HFD for 8 weeks and H&E staining of Liver section. Total fat contents were analyzed in liver tissues. (B) Liver weight of WT and TG mice fed HFD were measured after 8 weeks. Data shown represent the mean ± SEM and * denotes $P<0.05$.

PHF2 overexpression reduces lipid synthesis in hepatocytes.

To further investigate that PHF2 prevents the development of non-alcoholic fatty liver disease, I performed serum analysis from each feeding group of WT and TG mice. Relatively lower level of AST and ALT activity were measured in TG mice. In addition, serum cholesterol content was slightly lower. (Fig 3A) However, all data from serum analysis was statistically non-significant. To elucidate the mechanism by which PHF2 prevents fatty liver, the expression of genes involved in lipid metabolism were measured. The mRNA level of fatty acid synthase (FAS) and Acetyl-coenzyme A carboxylase (ACC) were markedly reduced in HFD fed TG mice. The mRNA level of SREBP1c was not significantly lowered in TG mice. In protein level, SREBP1 and FAS were significantly reduced in PHF2 over-expressing mice. These findings suggest that overexpression of PHF2 reduces the genes involved in lipid metabolism, resulting in a protective effect from steatosis.

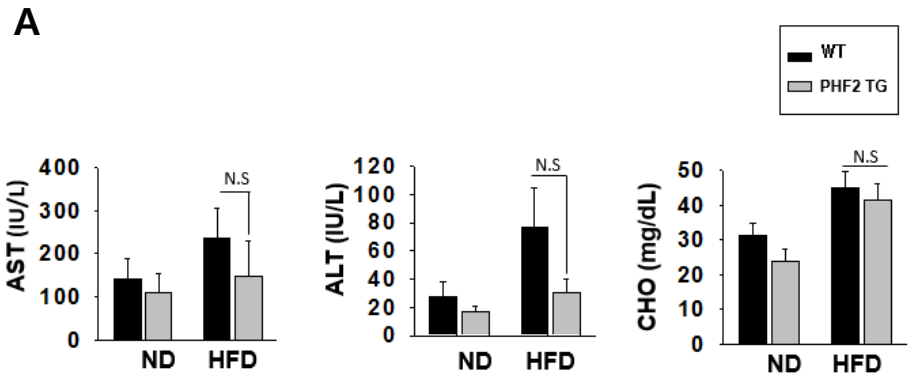


Figure 3. TG liver might be protected from lipotoxicity. Serum AST, ALT and cholesterol level were measured.

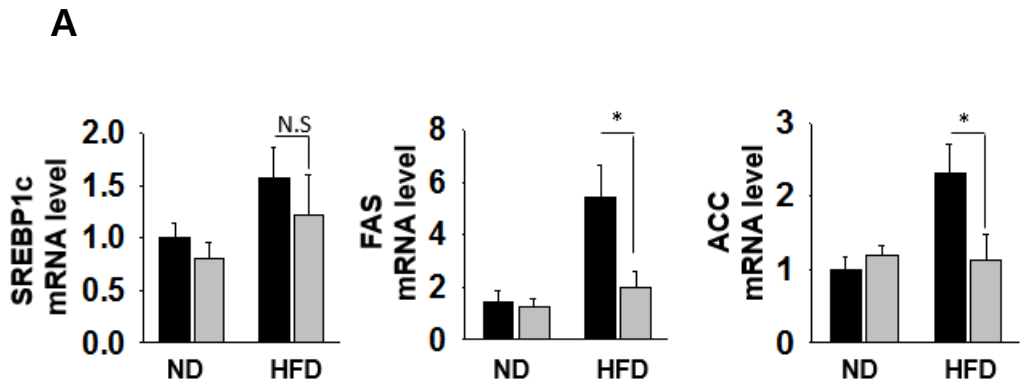


Figure 4. PHF2 overexpression down-regulates the expression of lipogenic genes. (A) RNAs extracted from liver tissues were subjected to RT-qPCR to check the expression of SREBP1c, FAS and ACC1 mRNAs. The mRNA levels (mean±SEM, n=3) are shown as relative values to 18S RNA levels. *p<0.05.

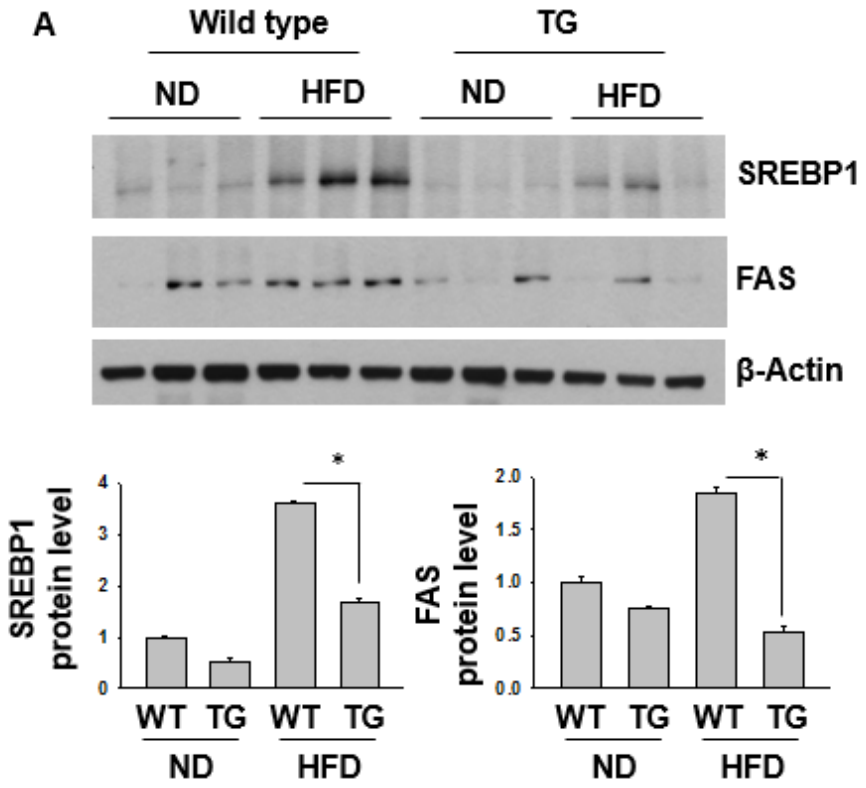
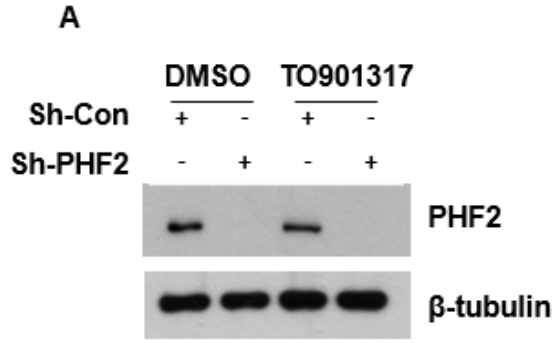
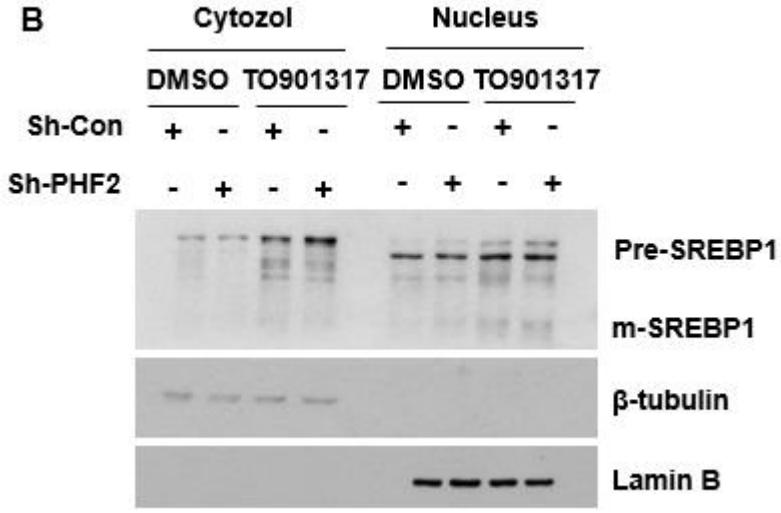


Figure 5. Protein levels of lipogenic genes were reduced in livers of PHF2 TG mice (A) Protein expression of SREBP1, FAS were analyzed by western blotting. The band intensities (mean + SD, n=3) were calculated using ImageJ and plotted in the bottom.

PHF2 knock down expression upregulates lipid metabolism related genes in hepatocyte cell line.

Next, I investigated whether PHF2 regulates genes involved in hepatic lipid metabolism in vitro. HepG2 cell line was infected with Lenti-viral shPHF2 and Lenti-Luci as a control vector. The cells were treated with TO901317, LXR agonist as an inducer of lipid metabolism for 24hours. PHF2 Knock down markedly induced mRNA of SREBP1, FAS and SCD1. In addition, the protein level of SREBP1 in cytosol and nucleus was also increased while PHF2 was silenced. The protein level of FAS followed same course with SREBP1. These results further supported my hypothesis that PHF2 represses hepatic lipid metabolism.


A
DMSO
TO901317
Sh-Con
+ - + -
Sh-PHF2
- + - +
PHF2
β-tubulin


B
Cytozol
Nucleus
DMSO TO901317 DMSO TO901317
Sh-Con
+ - + - + - + -
Sh-PHF2
- + - + - + - +
Pre-SREBP1
m-SREBP1
β-tubulin
Lamin B

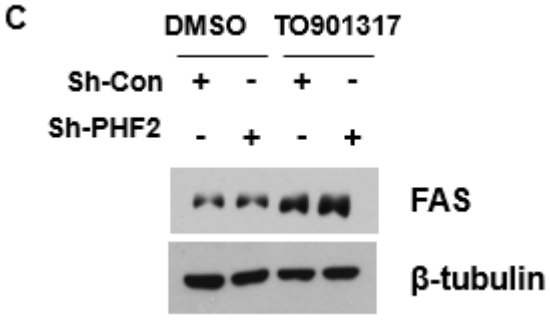

C
DMSO
TO901317
Sh-Con
+ - + -
Sh-PHF2
- + - +
FAS
β-tubulin

Figure 6. Knock down of PHF2 in HepG2 increase protein levels of lipogenic genes. (A) HepG2 stably expressing Sh-control (Sh-Con) or Sh-PHF2 (Sh-PHF2) were stimulated with 1 μM of TO901317 (LXR agonist) for 24 h, and then (B) Protein levels of PHF2 pre-SREBP1 and C-SREBP1 were analyzed by western blotting from nuclear and cytozol extracts. (C) Whole Cell lysates were subjected to western blotting using FAS antibodies.

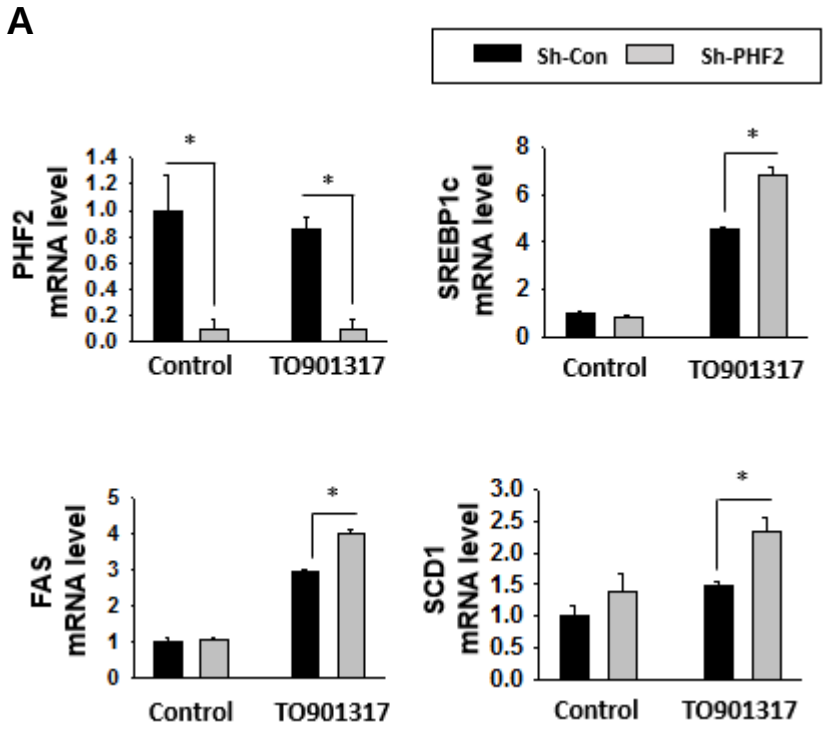


Figure 7. Knock down expression of PHF2 in HepG2 up-regulated SREBP1c and its down-stream genes. (A) HepG2 stably expressing sh-control (Sh-Con) or sh-PHF2 (Sh-PHF2) were stimulated with 1 μM of TO901317 (LXR agonist) for 24 h. RT-qPCRs were performed to evaluate the expression of PHF2, SREBP1c, FAS and SCD1 mRNAs.

PHF2 directly interacts with SREBP1c.

PHF2 is known to interact with transcription factors such as NF-κB, ARID5 and C/EBPs as a histone demethylase. (Baba et al., 2011; stender et al., 2012; Lee et al., 2014) Based upon such knowledge, I hypothesize that PHF2 might interact with SREBP1, a putative lipogenic transcription factor in liver. (Fig 8 A) Thus, I performed co-immunoprecipitaion analysis PHF2 with endogenously expressed SREBP1. PHF2 was found to have direct interaction with SREBP1.

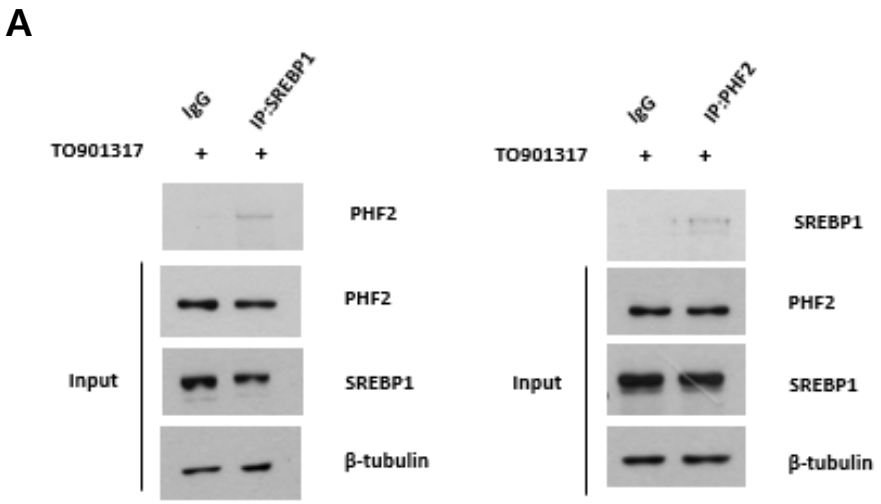


Figure 8. PHF2 directly interacts with SREBP1. (A) HepG2 were stimulated with 1 μM of TO901317 for 24 h. Cell lysates were subjected to immunoprecipitation with anti-SREBP1 or anti-PHF2 antibody, and the precipitated protein were analyzed by immunoblotting.

Table 1

Nucleotides sequences of primers used in experiments

RT-PCR primer	
PHF2_For	CCTGCTGGAGGCATTCAAAG
PHF2_Rev	CACGATCGGAAAGCACCATT
SREBP1_For	GGAGGGGTAGGGCCAACGGCCT
SREBP1_Rev	CATGTCTTCGAAAGTGCAATCC
FAS_For	ACAGGGACAACCTGGAGTTCT
FAS_Rev	CTGTGGTCCCACTTGATGAGT
shRNA oligo sequence	
Con_sh_Fwd	CCGGCGUGAUCUUCACCGACAAGAUCUCGAGAUCUUGUCGGUGAAGAUCACGUUUUU
Con_sh_Rev	AAUUAAAAACGUGAUCUUCACCGACAAGAUCUCGAGAUCUUGUCGGUGAAGAUCACG
PHF2_sh01_Fwd	CCGGGAGCUGAAGAUAGACGAGUUUCUCGAGAAACUCGUCUAUCUUCAGCUCUUUUU
PHF2_sh01_Rev	AAUUAAAAAGAGCUGAAGAUAGACGAGUUUCUCGAGAAACUCGUCUAUCUUCAGCUC

DISCUSSION

Through experiments, it was possible to know that PHF2 plays an essential role in the progression of NAFLD. SREBP1c is known as a key transcription factor related to hepatic steatosis. In this present study, I found out that jumonji histone demethylase, PHF2 acts as a negative regulator that attenuates liver steatosis in vivo study. PHF2 overexpressed TG mice markedly reduced insulin resistance and lipid accumulation in liver tissues, even though the weight of liver was not different between the WT and the TG. Reduced expression level of lipogenic genes from the liver tissues of TG mice further supports these results. However, the mRNA level of SREBP1c in HFD fed TG mice was not statistically significant whereas protein level was markedly decreased. This result showed me a clue that SREBP1 might be regulated in post-transcriptional manner rather than in transcriptional level.

Blood analysis was not statistically different between WT and TG. These results might be influenced by variation between each individual mouse.

PHF2 knock-down in the HepG2 liver cell line, dramatically

increased the expression of major genes related to fatty acid synthesis. Interestingly, the mRNA level of SREBP1c increased in vitro. It has been reported that SREBP1c autoregulates itself. (Amemiya-Kudo M, 2000) Thus, the effect of autoregulation of SREBP1 might more stand out in response to the LXR agonist, TO901317. We also demonstrated direct interaction between PHF2 and SREBP1c. On the basis of these findings, we identified PHF2 as a negative co-regulator of de novo synthesis in hepatocytes through the interaction with SREBP1c.

My data shows that PHF2 represses adipogenic gene transcription independent from its demethylase activity. PHF2 is generally reported for its involvement in facilitating various transcription factors driven gene expression by demethylating H3K9. PHF2 activates chondrocyte differentiation related to Sox9 target genes by stimulating H3K9me2 demethylation of these regions. (Kenji, 2013) In adipogenesis, PHF2 binds with CEBPα and CEBPδ and positively regulates the adipogenic gene expression during adipocyte differentiation by demethylation activity. (Kyoung-Hwa el al. 2014) It also has been reported that PHF2 represses ribosomal RNA gene transcription by antagonizing PHF8 activity and recruiting

methyltransferase Suv39H1. (Guang et al. 2014) The reason for the differences between previous study and our study needs to be more elucidated, but it may lie on the organ specific variation. Thus, my data might be consistent with the results of Guang el al. and suggests PHF2 may play a role as a repressor.

In summary, we identified that the histone demethylase PHF2 acts as a negative co-regulator through the interaction with the putative NAFLD related transcription factor, SREBP1c. These findings implicate that specific PHF2 activator may be useful in pharmaceutical intervention of NAFLD.

REFERENCES

Baba A, Ohtake F, Okuno Y, Yokota K, Okada M, Imai Y, Ni M. PKA-dependent regulation of the histone lysine demethylase complex PHF2-ARID5B. Nat Cell Biol 2011;13:668-675.

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

Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 2006;44:865-873.

Fan JG. Epidemiology of alcoholic and nonalcoholic fatty liver disease in China. J Gastroenterol Hepatol 2013;28 Suppl 1:11-17.

Foretz M, Pacot C, Ugail I, Lemarchand P, Guichard C, Lie ovre XL, Bertheier-lubrano C. ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. MCB 1999;19:3760-3768.

Fortschegger K, Shiekhattar R. Plant homeodomain fingers form a helping hand for transcription. Epigenetics 2011;6:4-8.

Hata K, Takashima R, Amano K, Ono K, Nakanishi M, Yoshida M, Wakabayashi M. Arid5b facilitates chondrogenesis by recruiting the histone demethylase Phf2 to Sox9-regulated genes. Nat Commun 2013;4:2850.

Ikarashi N, Toda T, Okaniwa T, Ito K, Ochiai W, Sugiyama K. Anti-Obesity and Anti-Diabetic Effects of Acacia Polyphenol in Obese Diabetic KKAy Mice Fed High-Fat Diet. Evid Based Complement Alternat Med 2011;2011:952031.

Kim HJ, Park JW, Lee KH, Yoon H, Shin DH, Ju UI, Seok SH.

Plant homeodomain finger protein 2 promotes bone formation by demethylating and activating Runx2 for osteoblast differentiation. Cell Res 2014;24:1231-1249.

Lazo M, Hernaez R, Bonekamp S, Kamel IR, Brancati FL, Guallar E, Clark JM. Non-alcoholic fatty liver disease and mortality among US adults: prospective cohort study. BMJ 2011;343:d6891.

Lee JH, Friso S, Choi SW. Epigenetic mechanisms underlying the link between non-alcoholic fatty liver diseases and nutrition. Nutrients 2014;6:3303-3325.

Lee KH, Ju UI, Song JY, Chun YS. The histone demethylase PHF2 promotes fat cell differentiation as an epigenetic activator of both C/EBPalpha and C/EBPdelta. Mol Cells 2014;37:734-741.

M L, Bindesbll C, I H. The Role of Liver X Receptor in Hepatic de novo Lipogenesis and Cross-Talk with Insulin and Glucose Signaling. Intech 2013.

Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003;37:917-923.

Okuno Y, Ohtake F, Igarashi K, Kanno J, Matsumoto T, Takada I, Kato S. Epigenetic Regulation of Adipogenesis by PHF2 Histone Demethylase. Diabetes 2013;62:1426-1434.

Sakai J, Rawson RB, Espenshade PJ, Cheng D, Seegmiller AC, Goldstein JL, Brown MS. Molecular Identification of the Sterol-Regulated Luminal Protease that Cleaves SREBPs and Controls Lipid Composition of Animal Cells. Mol Cell 1998;2:505-514.

Shi G, Wu M, Fang L, Yu F, Cheng S, Li J, Du JX. PHD finger protein 2 (PHF2) represses ribosomal RNA gene transcription by antagonizing PHF finger protein 8 (PHF8) and recruiting methyltransferase SUV39H1. J Biol Chem 2014;289:29691-29700.

Shimomura I, Matsuda M, Hammer RE, Bashmakov Y, Brown MS,

Goldstein JL. Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ob/ob mice. Mol Cell 2000;6:77-86.

Stender JD, Pascual G, Liu W, Kaikkonen MU, Do K, Spann NJ, Boutros M. Control of proinflammatory gene programs by regulated trimethylation and demethylation of histone H4K20. Mol Cell 2012;48:28-38.

Wen H, Li J, Song T, Lu M, Kan PY, Lee MG, Sha B. Recognition of histone H3K4 trimethylation by the plant homeodomain of PHF2 modulates histone demethylation. J Biol Chem 2010;285:9322-9326.

국문 초록

비알콜성 지방간은 간세포 내 과도한 지방 축적에 의해 형성된다. 처음에는 가벼운 지방축적으로 시작되어서 간염, 간 경변, 결국 간암까지 발전할 수 있게 된다. 히스톤 수식화는 후생유전학적 조절 중 하나로 생물학적 조절에 큰 역할을 하고 있다고 알려져 있다. Plant homeodomain finger protein 2 (PHF2) 는 쥬몬지 히스톤 탈메틸화 효소로 H3K9-Me2 를 탈메틸화 시킨다고 알려져 있다. PHF2 는 선행 연구들에서 지방대사와 관련된 전사인자를 조절한다고 보고되고 있다. 본 연구에서는 비알콜성 지방간 형성에서의 PHF2 역할을 규명하고자 하였다.

WT 쥐 및 PHF2 과발현 쥐를 12 주간 고지방식을 먹임으로써 지방간 형성을 유도하였다. 이 후 지방간 형성 정도와 혈장 분석을 실시 하였으며, 지방 대사와 관련된 유전자들의 단백질 및 유전자 발현의 변화를 조사하였다. HepG2 간세포 주에 Lenti-virus 를 이용하여 PHF2 발현을 억제함으로써 나타나는 변화를 관찰하였다.

PHF2 가 과발현된 쥐들은 WT 쥐들 보다 지방간이 현저히 적게 형성되어있는 것을 확인 할 수 있었으며, 인슐린저항성 또한 현저히 낮아져 있었다. 유전자 발현 양상을 조사한 결과, PHF2 과발현 쥐의 간에서 지방 형성에 관련된 유전자들의 발현이 낮아져 있는 것을 확인할 수 있었다. 정상 세포 및 PHF2 가 억제된 HepG2 세포에 지방 합성 촉

진을 유도한 다음. 관련된 유전자들의 발현을 확인한 결과 그 발현이 증가 되었다. 또한 PHF2 가 SREBP1 와 직접적으로 결합함을 확인 할 수 있었다.

PHF2 는 SREBP1 과 결합을 통해 비알콜성 지방간 형성에 억제인자로 작용함을 알 수 있었다. 따라서 PHF2 는 비알콜성 지방간 치료제 개발의 표적으로서 기여할 수 있을 것으로 사료된다.

주요어 : 비 알콜성 지방간, Non-alcoholic fatty liver disease, PHF2, SREBP1

학 번 : 2013-23529