Master's Thesis

HDAC1 Interacts with c-Myc to Increase Angiotensinogen Transcription in Obesity-Induced Hypertension

Department of Molecular Medicine
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1. Introduction

The rapid growth of obesity worldwide has led to the establishment of obese hypertension as a major cause of various complications and premature death (1). Moreover, obesity is a cause of resistant hypertension, which constitutes 10 - 15% of hypertension cases. Individuals with obese hypertension are less likely to respond to appropriate blood pressure (BP) control measures despite the use of at least three antihypertensive drugs including inhibitors for angiotensin II (Ang II) (2), even though renin-angiotensin-aldosterone system activation represents a leading cause of the disease (3).

Epigenetics provides a new approach for hypertension diagnosis, prognosis, and drug discovery (4). Previous report suggested that a broad spectrum histone deacetylase (HDAC) inhibitor, valproate, inhibits high-fat diet (HFD)-induced expression of renal angiotensinogen, a precursor of Ang II, ameliorating obese hypertension in mice. These findings revealed the important role of HDAC1 in angiotensinogen expression (5). However, the mechanisms and transcriptional regulation targets of HDAC1 in hypertension remain to be elucidated. Moreover, considering the distinct tissue distribution and cellular localization of individual HDACs, selective HDAC inhibitors, that possess better therapeutic index and fewer adverse effects, are necessary (6).

A transcription factor c-Myc, a well-known proto-oncogene, was selected as a target of this study by following lines of evidence. First, it has been shown that HFD fuels prostate cancer progression by amplifying the Myc signal (7). Second, HDAC inhibitor treatment downregulates c-Myc to promote cancer cell apoptosis in an acute myeloid leukemia model (8). Third, c-Myc interacts with HDAC1 at the

HIV-1 promoter and blunts HIV promoter expression (9).

FK228 inhibits HDAC1 and HDAC2 selectively and strongly (10). It was approved by the United States Food and Drug Administration for the treatment of cutaneous T-cell lymphoma and peripheral T-cell lymphoma (11,12). FK228 showed a strong anti-tumor effect against tumor xenografts in mice concomitant with the suppression of c-Myc and induction of p21 (13). However, the potential for the selective HDAC1 and HDAC2 inhibitor FK228 to be repurposed as a potent anti-hypertensive therapy for obese hypertension through the inhibition of c-Myc has not yet been evaluated. This study investigated the role of HDAC1 and c-Myc in HFD-induced angiotensinogen transcription and tested the effect of FK228 on this mechanism.

2. Materials and Methods

2.1. Animal Experiments:

All the animal experiments were conducted according to the guidelines of the National Institutes of Health for the Care and Use of Laboratory Animals. The experimental protocols (KM-2017-34R1 and KM-2019-28R1) were approved by the Institutional Animal Care and Use Committee at Keimyung University School of Medicine and the study was complied with all ethical regulations. Eight-week-old male C57BL/6 mice (C57BL/6NHsd, Koatech, Korea) were used in this study. The mice were randomly assigned to receive either a control, normal diet (ND) or HFD, containing 10% or 60% Kcal from fat, respectively (Harlan Laboratories, Madison, WI, USA). When the HFD-fed mice reached a hypertensive phase of over 140 mmHg systolic BP, they were administered FK228 (Abcam, Trumpington, Cambridge, UK) or 10058-F4 (Selleckchem, Houston, TX, USA) with vehicle (Veh). FK228 was used at the dose of 100 μg/kg body weight (BW) per day via intraperitoneal injection for 17 days. 10058-F4 was given at the dose of 20 mg/kg BW per day via intraperitoneal injection for 15 days. Mice were anesthetized with 50 mg/kg BW of pentobarbital sodium via intraperitoneal injection. Kidneys were frozen in liquid nitrogen and stored at −80 °C until analysis or embedded in paraffin wax.

2.2. Body Weight Measurement:

BW of the mice fed ND or HFD were measured using a scale (AND

KC-300, AND KOREA, Seoul, Korea).

2.3. Blood Pressure Measurement:

BP of mice were measured using a noninvasive tail-cuff system (CODA mouse rat tail-cuff system, Kent Scientific Corporation, Torrington, CT, USA) according to the manufacturer's instructions. The values after 10 consecutive readings were averaged per mouse (14).

2.4. Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) Analysis:

The total RNA of the kidneys or human embryonic kidney 293 (HEK 293) cells lysates were extracted using PureHelix RNA extraction solution (Nanohelix, Seoul, Korea). Subsequently, 1 μg of RNA were used for cDNA synthesis by using the DiaStar RT Kit (SolGent, Daejeon, Korea). qRT-PCR was performed using LightCyclerR 480 SYBR Green I Master mix (Roche, Basel, Basel-Stadt, Switzerland) and the real-time PCR system (LightCycler 96, Roche, Basel, Basel-Stadt, Switzerland) (Table 1).

2.5. HDAC1 and HDAC2 Activity Assays:

HDAC1 and HDAC2 activities in the kidneys were measured using HDAC1 (BioVision, Milpitas, CA, USA) and HDAC2 (BioVision, Milpitas, CA, USA) activity assay kits, respectively, following the manufacturer's instructions.

2.6. Human Embryonic Kidney 293 Cell Culture and Gene Silencing:

HEK 293 (293, Korean Cell Line Bank, Korea) cells were grown in Dulbecco's Modified Eagle's Medium (Weljin, Taipei, Fujian, China) with fetal bovine serum (Gibco, Grand Island, NY, USA) and streptomycin/penicillin (Weljin, Taipei, Fujian, China) at 37 °C in a humidified atmosphere containing 5% carbon dioxide. For silencing HDAC1 expression, HEK 293 cells at 80% confluence were transfected with 25 nmol/L HDAC1 small interfering RNA or 25 nmol/L scrambled small interfering RNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) for 48 h following the manufacturer's instructions.

2.7. Subcellular Fractionation:

Kidneys were fractionated using a NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The acquired fractions were used for western blot.

2.8. Western Blot Analysis:

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) samples were prepared with kidney nuclear and cytoplasmic fractions. Total protein concentration was measured using a BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA)

and extractions were diluted with 5X SDS-PAGE loading buffer (60 mmol/L Tris-HCl, pH 6.8, 50% glycerol, 2% SDS, 0.1% bromophenol blue, 5% 2-mercaptoethanol) and heated at 98 °C for 5 min, then stored at −20 °C until use. The samples were electrophoresed on 10% polyacrylamide gels with 0.1% SDS, transferred onto nitrocellulose membranes, and then subjected to immunoblotting with antibodies. Immune-reactive bands were visualized using a chemiluminescence reagent (Thermo Fisher Scientific, Waltham, MA, USA). Signal intensities were quantified using the image analysis software ImageJ version 1.52a. Primary antibodies used included those against HDAC1 (Santa Cruz Biotechnology, Dallas, TX, USA), HDAC2 (Santa Cruz Biotechnology, Dallas, TX, USA), c-Myc (Invitrogen, Carlsbad, CA, USA), GAPDH (Novus Biologcials, Centennial, CO, USA), and Histone H1 (Santa Cruz Biotechnology, Dallas, TX, USA). Horseradish-peroxidase-conjugated secondary antibodies against rabbit (Bethyl Laboratories, Montgomery, TX, USA), mouse IgG (Bethyl Laboratories, Montgomery, TX, USA) or goat IgG (Bethyl Laboratories, Montgomery, TX, USA) were used.

2.9. Proximity Ligation Assay (PLA):

To test the interactions among HDAC1, HDAC2, and c-Myc in the kidneys, PLA was performed using a PLA kit (Sigma, St. Louis, MO, USA) according to the manufacturer's instructions. Periodate-lysine-paraformaldehyde-fixed kidneys were washed with phosphate-buffered saline, embedded in paraffin, and were cut into 3 μm sections using a microtome (HM 325 Rotary Microtome, Thermo Fisher Scientific, Waltham, MA, USA). The kidney sections were deparaffinized

with xylene and rehydrated with serial dipping in ethanol (100%, 95%, and 80%) and water. To unmask the antigen epitopes, the kidney sections were boiled in 10 mM sodium citrate buffer, pH 6.0, for 10 min. To block the activations of endogenous peroxidase, the kidney sections were treated with 3% hydrogen peroxide in phosphate-buffered saline at 4 °C for 30 min. The sections were blocked using the provided blocking buffer for 1 h at 37 °C. Sections were incubated with primary antibodies against HDAC1 (Santa Cruz Biotechnology, Dallas, TX, USA) and c-Myc (Abcam, Trumpington, Cambridge, UK) or HDAC2 (Santa Cruz Biotechnology, Dallas, TX, USA) and c-Myc (Abcam, Trumpington, Cambridge, UK) together at 4 °C overnight. After washing with wash buffer, anti-mouse MINUS (Sigma, St. Louis, MO, USA) and anti-rabbit PLUS (Sigma, St. Louis, MO, USA) secondary antibodies were applied along with the PLA probe at 37 °C for 1 h. After washing, ligation buffer was applied at 37 °C for 30 min. After washing, the amplification buffer was applied at 37 °C for 100 min in the dark. After washing, quenching of autofluorescence with 0.1% Sudan Black B in 70% ethanol solution was performed at room temperature for 20 min. After washing, sections were dried in the dark and mounted with mounting solution containing 4′,6-Diamidino-2-phenylindole (DAPI) (Sigma, St. Louis, MO, USA). The images were acquired using a confocal microscope (LSM5 EXCITER, Carl Zeiss Microscopy, GmbH, Jena, Germany).

2.10. Statistical Analysis:

The results were expressed as the means ± standard errors. Statistical significance was determined by Student's t-test to analyze differences between 2 groups. Differences between groups were considered

statistically significant at a p-value of < 0.05. Statistical tests were performed using Microsoft Excel 2016.

Table 1. List of Primers for Quantitative Real-Time Polymerase Chain Reaction Analysis

Gene	Forward	Reverse
Mouse Angiotensinogen	CTCGAACTCAAAGCAGGAGAG	GTAGATGGCGAACAGGAAGG
Mouse 18S rRNA	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
Human HDAC1	GGGATTGATGACGAGTCCTATG	GAGTCTGAGCCACACTGTAAG
Human Angiotensinogen	GATGTTGCTGCTGAGAAGATTG	AGTGGACGTAGGTGTTGAAAG
Human β-actin	CACTCTTCCAGCCTTCCTTC	GTACAGGTCTTTGCGGATGT

HDAC: histone deacetylase.

3. Results

3.1. High-Fat Diet Accelerated the Increase in Body Weight and Raised Blood Pressure:

Prior to differential feeding, BWs were not differ between ND and HFD groups. After 12 weeks, HFD group significantly increased BW compared to ND group (ND: 30.20 ± 1.06 and HFD: 42.82 ± 0.66 g) ($p < 0.001$, ND vs. HFD) (Figure 1A). In addition, whereas systolic BP of the ND and HFD groups were initially similar before diet, systolic BP after 12 weeks significantly changed (ND: 118 ± 2 and HFD: 145 ± 2 mmHg) ($p < 0.001$, ND vs. HFD) (Figure 1B). Similarly, diastolic BP had no difference between ND and HFD groups before diet, a significant changes were observed at 12 weeks (ND: 87 ± 3 and HFD: 113 ± 1 mmHg) ($p < 0.001$, ND vs. HFD) (Figure 1C).

3.2. Inhibitions of HDAC1 and HDAC2 by FK228 Ameliorated High-Fat Diet-Induced Hypertension:

FK228 did not affect either systolic or diastolic BP in the ND group, whereas both systolic BP (146 ± 1 to 122 ± 1 mmHg) ($p < 0.001$, day 1 vs. day 17) and diastolic BP (114 ± 2 to 99 ± 3 mmHg) ($p < 0.001$, day 1 vs. day 17) were lowered in the HFD group (Figure 2A&B). Conversely, BP levels were maintained in both the vehicle-treated HFD group (systolic BP: 152 ± 5 to 144 ± 7 mmHg and diastolic BP: 119 ± 2 to 116 ± 6 mmHg) and vehicle-treated ND group (Figure 2A&B).

3.3. Renal Angiotensinogen was Increased by High-Fat Diet and Decreased by FK228:

Angiotensinogen mRNA levels in the mouse kidneys were enhanced by HFD (2.41 ± 0.70 fold) ($p < 0.05$, ND Veh vs. HFD Veh). FK228 administration decreased the mRNA (0.70 ± 0.26 fold) ($p < 0.05$, HFD Veh vs. HFD FK228) levels of angiotensinogen (Figure 3).

3.4. High-Fat Diet Increased the Activities of HDAC1 and HDAC2, Which were Reversed by FK228:

HFD increased the activities of HDAC1 (2.43 ± 0.21 fold) ($p < 0.01$, ND Veh vs. HFD Veh) (Figure 4A) and HDAC2 (1.76 ± 0.05 fold) ($p < 0.01$, ND Veh vs. HFD Veh) (Figure 4B), whereas FK228 administration decreased the activities of HDAC1 (0.91 ± 0.14 fold) ($p < 0.01$, HFD Veh vs. HFD FK228) (Figure 4A) and HDAC2 (1.26 ± 0.04 fold) ($p < 0.001$, HFD Veh vs. HFD FK228) (Figure 4B) in the mouse kidneys.

3.5. Angiotensinogen Transcriptions Depended on HDAC1:

In HEK 293 cells, HDAC1 gene expressions were reduced by HDAC1 gene silencing compared to that of the control (Con) (49.9 ± 23.2% of Con) ($p < 0.05$, Con vs. knock down). HDAC1 gene silencing significantly reduced angiotensinogen gene expressions (21.4 ± 1.8% of Con) ($p < 0.01$, Con vs. knock down) (Figure 5).

3.6. High-Fat Diet Increased Nuclear Expressions of HDAC1, HDAC2, and c-Myc, Which were Reversed by FK228:

HFD increased the HDAC1 (3.76 ± 0.47 fold) ($p < 0.001$, ND Veh vs. HFD Veh) (Figure 6B), HDAC2 (2.68 ± 0.28 fold) ($p < 0.01$, ND Veh vs. HFD Veh) (Figure 6C), and c-Myc (4.29 ± 0.60 fold) ($p < 0.01$, ND Veh vs. HFD Veh) (Figure 6D) protein expressions in the nuclear fractions of mouse kidneys. Conversely, FK228 administration decreased the nuclear expressions of HDAC1 (1.74 ± 0.55 fold) ($p < 0.05$, HFD Veh vs. HFD FK228) (Figure 6B), HDAC2 (1.22 ± 0.42 fold) ($p < 0.05$, HFD Veh vs. HFD FK228) (Figure 6C), and c-Myc (1.99 ± 0.77 fold) ($p < 0.05$, HFD Veh vs. HFD FK228) (Figure 6D).

3.7. High-Fat Diet Increased the Interactions of c-Myc and HDAC1, Which were Reversed by FK228:

In the nuclei of mouse kidneys, PLA signals for HDAC1 and c-Myc in the HFD group increased compared to the ND group, which were decreased by FK228. PLA signals for the interactions between HDAC2 and c-Myc were not detectable (Figure 7).

3.8. c-Myc Inhibitor 10058-F4 Ameliorated High-Fat Diet-Induced Hypertension:

10058-F4 did not affect either systolic or diastolic BP in the ND

group, whereas it lowered both systolic BP (systolic BP: 149 ± 2 to 119 ± 2 mmHg) ($p < 0.001$, day 1 vs. day 15) and diastolic BP (115 ± 3 to 92 ± 1 mmHg) ($p < 0.001$, day 1 vs. day 15) in the HFD group (Figure 8A&B). Conversely, BP levels were maintained in both the vehicle-treated HFD group (systolic BP: 149 ± 3 to 146 ± 3 mmHg and diastolic BP: 117 ± 3 to 114 ± 2 mmHg) and vehicle-treated ND group (Figure 8A&B).

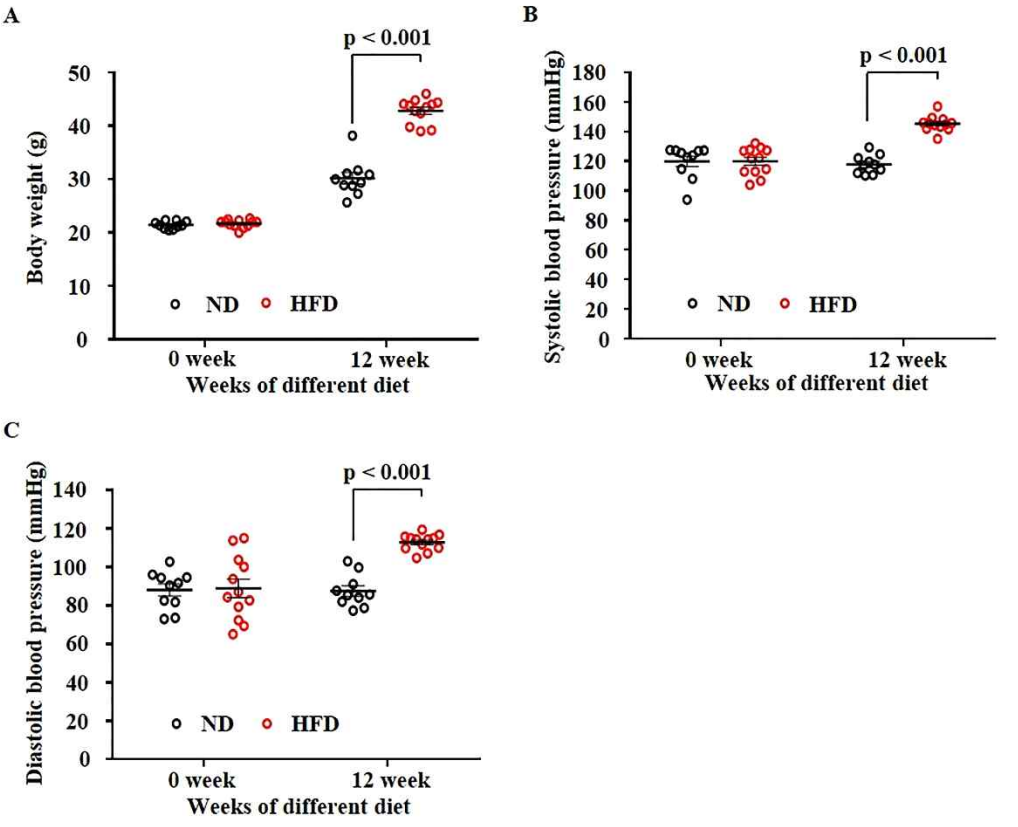

Figure 1. Changes in body weight and blood pressure by high-fat diet. Graphs demonstrate body weight (A), systolic blood pressure (B), and diastolic blood pressure (C) measured before and after 12 weeks of diet regimens. Results were expressed as the means ± standard errors (n = 10 mice per group). ND: normal diet; HFD: high-fat diet.

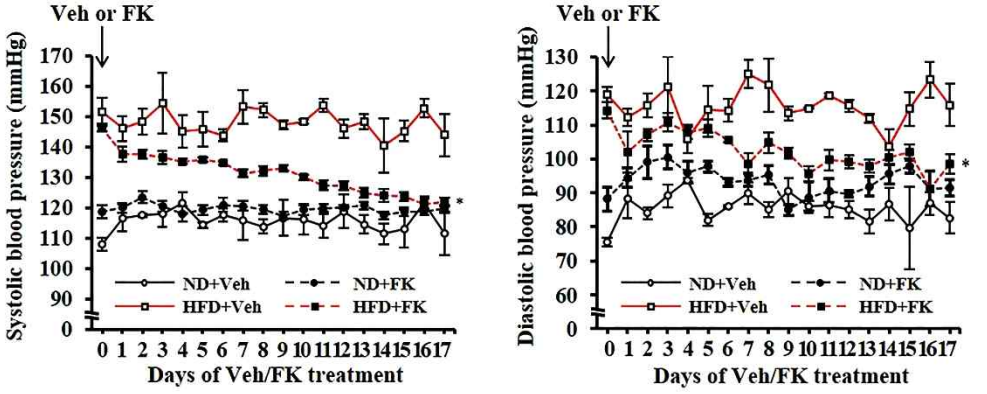

Figure 2. Effects of high-fat diet and FK228 on blood pressure. Graphs demonstrate systolic blood pressure (A) and diastolic blood pressure (B) measured during vehicle or FK228 (100 μg/kg body weight per day) administration after the 12 weeks of diet regimens. Results were expressed as the means ± standard errors (n = 5 mice per group). ND: normal diet; HFD: high-fat diet; Veh: vehicle; FK: FK228. *: $p < 0.001$ day 0 vs. day 17.

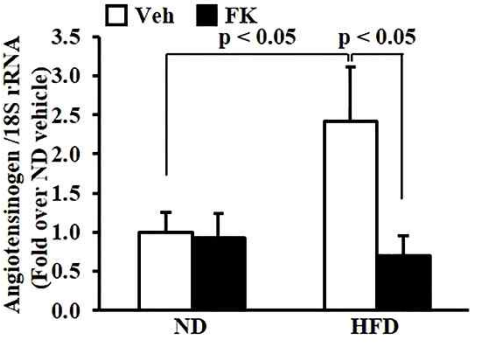

Figure 3. Effects of high-fat diet and FK228 on the expressions of angiotensinogen in mouse kidneys. Graph demonstrates the mRNA levels of angiotensinogen in the kidneys of ND- or HFD-fed mouse after vehicle or FK228 administration. Result was expressed as the mean ± standard error (n = 3 mice per group). ND: normal diet; HFD: high-fat diet; Veh: vehicle; FK: FK228.

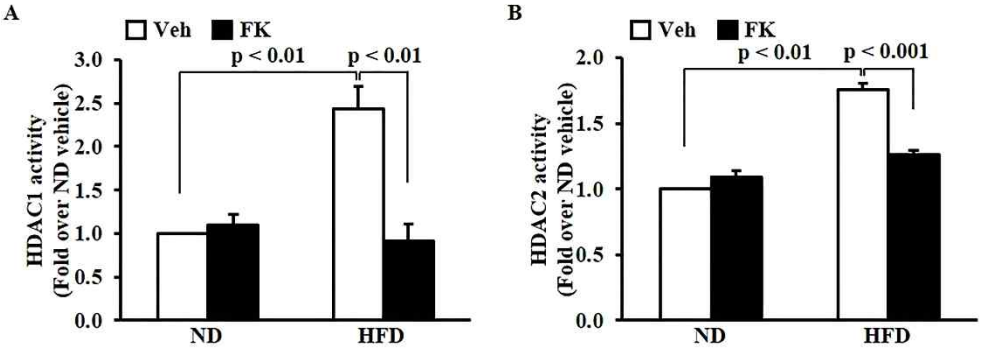

Figure 4. Effects of high-fat diet and FK228 on the activities of HDAC1 and HDAC2 in mouse kidneys. Activities of HDAC1 (A) and HDAC2 (B) in the kidneys of ND- or HFD-fed mouse after vehicle or FK228 administration. Results were expressed as the means ± standard errors (n = 3 mice per group). ND: normal diet; HFD: high-fat diet; Veh: vehicle; FK: FK228; HDAC: histone deacetylase.

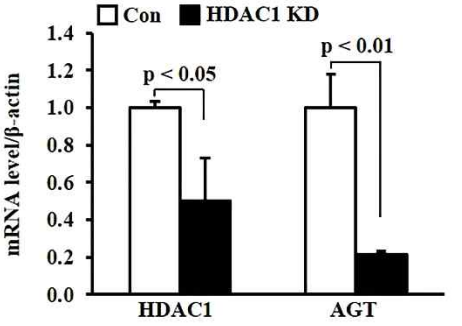

Figure 5. Effect of HDAC1 gene silencing on the expressions of angiotensinogen. The mRNA levels of HDAC1 and angiotensinogen in cultured HEK 293 cells upon silencing HDAC1. Result was expressed as the mean ± standard error (n = 3 mice per group). HEK 293: Human embryonic kidney 293; Con: control; KD: knockdown; HDAC: histone deacetylase; AGT: angiotensinogen.

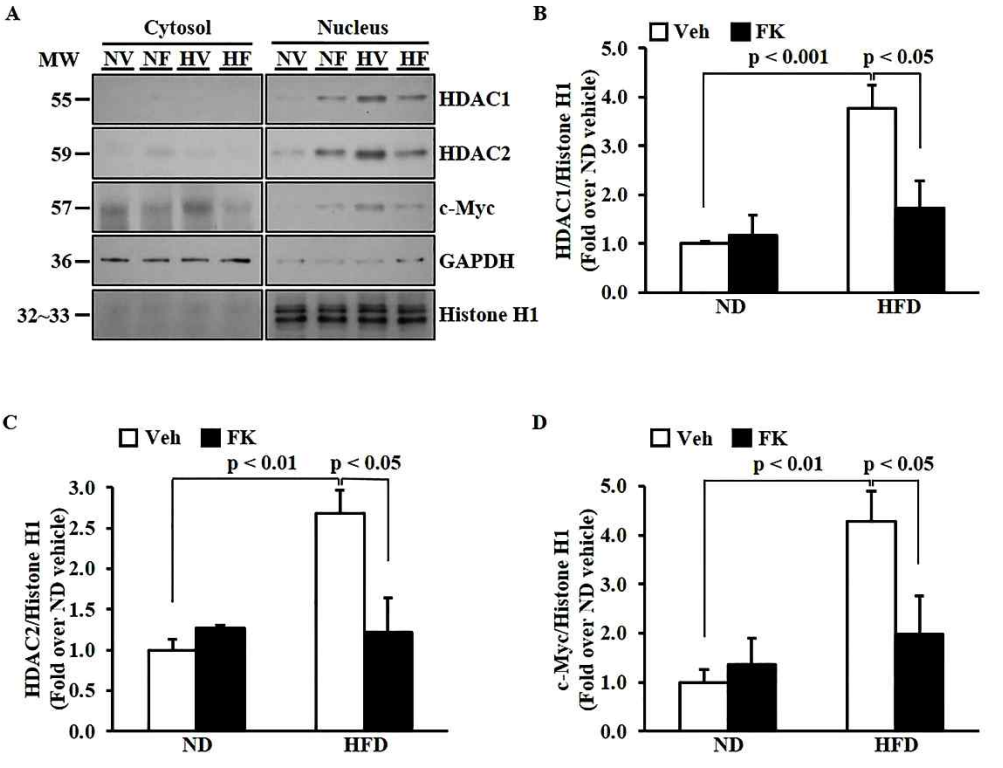

Figure 6. Effects of high-fat diet and FK228 on the cytoplasmic and nuclear expressions of HDAC1, HDAC2, and c-Myc in mouse kidneys. (A), Representative western blots for HDAC1, HDAC2, c-Myc, GAPDH, and Histone H1 in cytoplasmic and nuclear fractions. Protein expressions of HDAC1 (B), HDAC2 (C), and c-Myc (D) in the nuclear fractions of ND- or HFD-fed mouse following vehicle or FK228 administration. Results were expressed as the means ± standard errors (n = 3 mice per group). NV: normal diet with vehicle administration; NF: normal diet with FK228 administration; HV: high-fat diet with vehicle administration; HF: high-fat diet with FK228 administration; MW: molecular weight (kDa); ND: normal diet; HFD: high-fat diet; Veh: vehicle; FK:

FK228; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase; HDAC: histone deacetylase.

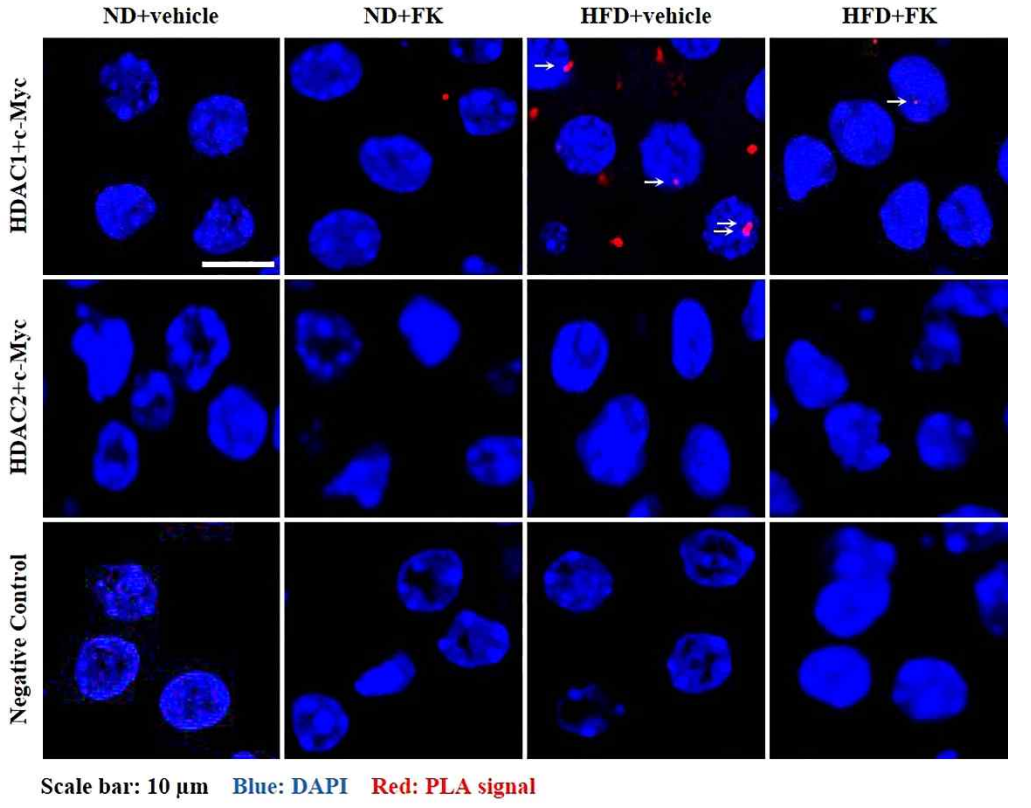

Figure 7. Effects of high-fat diet and FK228 on the interactions of HDAC1 and c-Myc or HDAC2 and c-Myc in mouse kidneys. Representative PLA results of interactions of HDAC1 and c-Myc or HDAC2 and c-Myc in ND- or HFD-fed mouse kidneys following vehicle or FK228 administration. Arrows pointing the red signals indicate the protein-protein interactions in nuclei of mouse kidneys. DAPI (blue) indicates nuclei. Staining only with secondary antibody probes was used as a negative control for PLA. Scale bar: 10 μm. ND: normal diet; HFD: high-fat diet; Veh: vehicle; FK: FK228; HDAC: histone deacetylase; PLA: proximity ligation assay; DAPI: 4′,6-Diamidino-2-phenylindole.

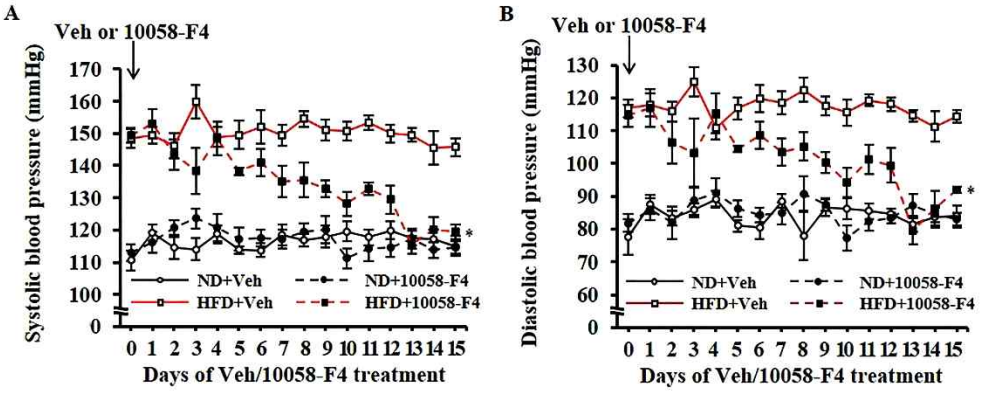

Figure 8. Effects of high-fat diet and 10058-F4 on blood pressure. Systolic blood pressure (A) and diastolic blood pressure (B) were measured during vehicle or 10058-F4 (20 mg/kg body weight per day) administration. Results are expressed as the means ± standard errors (n = 6 mice per group). Veh: vehicle; ND: normal diet; HFD: high-fat diet. *: $p < 0.001$ day 0 vs. day 15.

4. Discussion

The results of the present study demonstrate that HFD-induced hypertension can be attributed to HDAC1/c-Myc axis activation and consequent renal angiotensinogen expression. HFD activated HDAC1, resulting in binding of HDAC1 with c-Myc, consequently increasing angiotensinogen transcription in the kidney. The selective HDAC1 and HDAC2 inhibitor FK228 inhibited HDAC1 activation, HDAC1/c-Myc complex formation, angiotensinogen expression, and ultimately hypertension induced by HFD. In addition, 10058-F4 also lowered blood pressure by inhibiting c-Myc in HFD-induced hypertension.

Angiotensinogen is primarily produced by hepatic cells and secreted into the circulation to regulate plasma angiotensin I and II levels (15,16). However, plasma angiotensinogen rarely filters across the glomerular membrane because of its molecular size. Accordingly, in recent years, interest in the role of the renin - angiotensin system in the pathophysiology of hypertension has focused on the function of local renin - angiotensin systems in specific tissues including the kidney (17). In particular, increases have been reported in both mRNA and protein angiotensinogen levels in the kidney in multiple models of hypertension such as Dahl salt-sensitive hypertensive rats, spontaneously hypertensive rats, and various kidney diseases (18). Notably, angiotensinogen expression in the kidney is independent of that in hepatic cells (17) and dysregulation of the kidney system is now considered as a cause of the increased kidney angiotensin II level observed in hypertension (19). Moreover, previous report suggested that increased renal angiotensinogen plays a critical role in HFD-induced hypertension (5,20) and can be ameliorated by inhibition of HDAC1

using the broad-spectrum HDAC inhibitor valproate (5). Consistent with these findings, the present study revealed that the HDAC1 and HDAC2 selective inhibitor FK228 (21), which was originally developed as an anti-cancer agent (12), effectively lowered both systolic and diastolic BP associated with renal angiotensinogen elevated by HFD treatment. Thus, the data highlight a novel possibility of repurposing FK228 for pharmacological antihypertensive regimens.

The enhanced transcription of angiotensinogen following HFD is attributed to enhanced binding of GR, CEBP-β, and STAT3 in hepatic and adipose tissues (22). However, the transcription factors that are responsible for the transcriptional regulation of angiotensinogen in the kidney have not been studied. The present study found that the transcription factor c-Myc bound with HDAC1 and enhanced the transcription of angiotensinogen. This interrelationship is consistent with a prior report that c-Myc interacts with HDAC1 at the HIV-1 promoter (9). This finding that c-Myc mediated HFD-induced hypertension was supported by the following lines of evidence. First, HFD fuels prostate cancer progression by amplifying the Myc transcriptional program (7). Second, the smooth muscle cell proliferation shown in spontaneously hypertensive rats is inhibited by the angiotensin converting enzyme inhibitor quinapril through the inhibition of c-Myc expression (23). Third, c-Myc also raises BP in an Ang II-dependent manner by upregulating renal G protein-coupled receptor kinase 4 (GRK4), which negatively regulates dopamin1-1-receptor (D1R). Inhibition of c-Myc activity restored coupling of D1R to adenylyl cyclase stimulation (24-26). Consistent with these findings, the present study revealed that upregulation of c-Myc occurred in HFD-induced hypertension, whereas treatment with the c-Myc inhibitor 10058-F4 effectively lowered both systolic and diastolic BP. Thus, these findings suggest that the

HDAC1/c-Myc regulatory axis is involved in multiple forms of hypertension including obese hypertension.

Although HDAC2 inhibition by FK228 attenuated HFD-induced hypertension, the mechanism remains to be unclear. HDAC1 and HDAC2 exhibit high homology (27) and, similar to HDAC1, HFD also increased the activity of HDAC2. In contrast, HDAC2 did not bound with c-Myc. Therefore, HDAC2 appears to mediate HFD-induced hypertension in a mechanism distinct from that of HDAC1. Further study regarding the role of HDAC2 in HFD-induced hypertension will therefore likely reveal additional target(s) for hypertension treatment.

The interplay between acetylation and ubiquitination determines the stability of c-Myc. Acetylation negatively regulates c-Myc ubiquitination and proteasomal degradation, although limited information is available regarding the specific acetylation sites (28). c-Myc can be targeted by protein deacetylases such as SIRT1, which interacts with and deacetylates c-Myc resulting in decreased c-Myc protein stability to ultimately suppress tumor growth (29). However, it was recently reported that HDAC inhibitor treatment induces the acetylation of c-Myc and decreases its overall protein levels, leading to anti-cancer effects in acute myeloid leukemia (8). Similar results were obtained in this study. c-Myc expression increased in parallel with the upregulation of HDAC1 in HFD-induced hypertension, whereas treatment with FK228 decreased c-Myc expression.

Together, these findings indicate that regulation of c-Myc by HDAC1 is a critical factor for HFD-induced angiotensinogen expression and hypertension. The present study suggests repurposing the anti-cancer agent FK228 to treat obese hypertension and highlights c-Myc as a novel therapeutic target for modulating HFD-induced renal angiotensinogen expression and hypertension.

5. Summary

The aim of this study was to investigate the mechanism of HDAC1 in HFD-induced angiotensinogen transcription and discover the effect of FK228 on this mechanism. Mice were fed either ND or HFD, followed by FK228 treatment. HFD-induced increase in HDAC1/c-Myc complex formation in the nucleus of mouse kidney enhanced angiotensinogen transcription resulting in hypertension. Inhibition of HDAC1 using selective HDAC1 and HDAC2 inhibitor attenuated HFD-induced hypertension, presenting it as a novel therapeutic option for hypertension. Inhibition of c-Myc supported these findings by lowering blood pressure raised by HFD. Thus, the present study offers HDAC1 and c-Myc as novel targets for obese hypertension.

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HDAC1 Interacts with c-Myc to Increase Angiotensinogen Transcription in Obesity-Induced Hypertension

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(Abstract)

Dysregulation of renal angiotensinogen is a critical factor for the development of hypertension. Previous study reported the effects of broad-spectrum histone deacetylase (HDAC) inhibitors on a high-fat diet (HFD) induced renal angiotensinogen expression and the importance of HDAC1 for this phenomenon. However, the underlying mechanisms remain to be elucidated. Present study revealed the roles of HDAC1 and HDAC2 in HFD-induced hypertension using a selective HDAC1 and HDAC2 inhibitor, FK228, and the mechanism by which HDAC1 regulates angiotensinogen transcription. Male C57BL/6 mice were fed for 12 weeks either a normal diet or HFD, followed by FK228 treatment. HFD induced hypertension by increased renal angiotensinogen

expression. HFD activated HDAC1 and HDAC2 in the kidney and FK228 reversed these effects. HDAC1 silencing in human embryonic kidney 293 cells decreased angiotensinogen expression. HFD increased nuclear expression of HDAC1, HDAC2, and a transcription factor c-Myc, whereas HDAC1 but not HDAC2 bound with c-Myc. FK228 decreased these effects, ameliorating hypertension. Supporting these findings, the treatment of 10058-F4, a c-Myc inhibitor, decreased HFD-induced hypertension. Collectively, HFD increased HDAC1 activity, recruiting the HDAC1/c-Myc complex, resulting in increased renal angiotensinogen expression and hypertension. FK228 ameliorated HFD-induced hypertension by inhibiting these effects.

비만에 의한 고혈압에서 히스톤 탈아세틸화 효소 1과 시믹의 상호작용에 의한 안지오텐시노겐 발현 증가 기전에 관한 연구

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(초록)

신장 안지오텐시노겐의 조절 장애는 고혈압의 발생에 중요한 요인이다. 고지방 식이로 유도한 고혈압에 관한 이전 연구에서는 히스톤 탈아세틸화 효소1이 신장의 안지오텐시노겐 발현에 중요함과 광범위 히스톤 탈아세틸화 효소 억제제의 효과가 보고되었다. 본 연구에서는 보다 근본적인 메커니즘을 설명하기 위하여, 선택적 탈아세틸화 효소1과 2의 억제제인 FK228을 사용하여 고지방식이로 유도한 고혈압에서 히스톤 탈아세틸화 효소1과 2의 역할과 히스톤 탈아세틸화 효소1이 안지오텐시노겐 전사를 조절하는 메커니즘을 연구하였다. 수컷 C57BL/6 쥐에게 12주 동안 일반식이 또는 고지방식이를 먹인 후 FK228을 투여하였다. 고지방식이는 신장 안지오텐시노겐 발현을 증가시켜 고혈압을 유발하였다. 고지방식이는 또한 신장에서 히스톤 탈아세틸화 효소1과 2를 활성화시켰고 FK228 투여 시 활성이 감소하였다. HEK 293세포에서 히스톤 탈아세틸화 효소1 유전자의 발현 억제는 안지오

텐시노겐 발현을 감소시켰다. 고지방식이는 신장의 핵에서 히스톤 탈아세틸화 효소1, 2 그리고 전사 인자 시믹의 발현을 증가시켰으며, 히스톤 탈아세틸화 효소1과 시믹의 결합을 증가시켰다. FK228의 투여는 이러한 현상을 감소시켜 고혈압을 개선하였다. 또한 시믹 억제제인 10058-F4의 투여는 고지방식이로 유도한 고혈압을 감소시켰다. 결론적으로, 고지방식이는 히스톤 탈아세틸화 효소1을 활성화하고 히스톤 탈아세틸화 효소 1과 시믹의 복합체 형성을 증가시켜 신장의 안지오텐시노겐 발현의 증가로 고혈압을 일으켰다. FK228은 이러한 현상을 억제함으로써 고지방식이로 유도한 고혈압을 개선하므로 비만성 고혈압 치료제로 사용될 수 있음을 제안한다.