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A novel upstream transcription factor 1 target gene *N4bp2l1* that regulates adipogenesis

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ABSTRACT

N4BP2l1, which is highly expressed in oral squamous cell carcinoma, is associated with poor prognosis. However, *N4bp2l1*'s role in adipogenesis remains unknown. We aimed to clarify the expression profile and transcriptional regulation of *N4bp2l1* to elucidate the functions underlying the role of *N4bp2l1* in adipocyte differentiation. Our results revealed that *N4bp2l1* mRNA expression increased in 3T3-L1 cells in a differentiation-dependent manner. To investigate the transcriptional regulation of *N4bp2l1*, the 2-kb 5′ region upstream of the mouse *N4bp2l1* promoter was cloned into a luciferase vector. Luciferase reporter assays indicated that USF1 induces the *N4bp2l1* promoter activity. Electrophoretic mobility shift and chromatin immunoprecipitation assays confirmed that USF1 directly binds to the Ebox in the *N4bp2l1* promoter. Furthermore, the expressions of adipocyte differentiation markers significantly decreased in *N4bp2l1*-knockdown cells compared with those in control cells. Our results demonstrated that *N4bp2l1* is a novel USF1 target gene that may be involved in adipogenesis regulation.

1. Introduction

Individuals with obesity have an increased risk of developing type 2 diabetes, atherosclerosis, hyperlipidemia, steatosis, and various types of cancer [1–3]. Adipogenesis is a process of progenitor cell differentiation and is controlled by the interplay of many transcription factors and complex molecular regulatory networks. For adipocyte differentiation, several groups of transcription factors regulating the key proteins need to be sequentially activated; this in turn induces gene expression that leads to adipocyte development. The key proteins include CCAAT/enhancer binding proteins (C/EBPs) and peroxisome proliferator-activated receptor gamma (PPARg) that coordinate the expression of genes that generate and maintain the adipocyte phenotype [4,5]. Over the years, many studies have been performed to identify the transcriptional processes and molecular mechanisms involved in adipocyte differentiation. However, more studies are required to identify novel regulators of adipogenesis and the appropriate targets for anti-obesity drugs for the treatment of obesity pathogenesis. The 3T3-L1 preadipocyte is a well-established model for investigating mechanisms underlying adipocyte proliferation, differentiation, and lipid metabolism and is appropriate for the identification of genes that regulate adipocyte physiology, including adipogenesis [6–8].

Upstream transcription factor 1 (USF1), a ubiquitously expressed helix–loop–helix leucine zipper transcription factor, recognizes the Ebox motif CACGTG [9] and regulates several proteins involved in lipid metabolism, including apolipoproteins apoA-II [10], apoA-V [11], apoC-III [12], apoE [13], hepatic lipase [14], ATP-binding cassette transporter-1 [15], fatty acid synthase [9,16], and hormone-sensitive lipase [17]. Moreover, genetic variants of *USF1* are associated with familial combined hyperlipidemia [16,17], increased cardiovascular disease risk [17], atherosclerotic lesions [18], and body mass index (BMI) variations [19].

The NEDD4-binding protein 2-like 1 gene (*N4BP2L1*) produces a full-length transcript with 5 exons and encodes a 243-amino-acid protein. This protein possesses several alternatively spliced isoforms that map to the human chromosome 13q13.1. *N4bp2l1* is highly expressed in nasopharyngeal carcinoma and oral squamous cell carcinoma [20], and *N4BP2L1* overexpression is reportedly correlated with nodal metastasis and poor outcomes in oral squamous cell carcinoma [20,21]. *N4bp2l1* has been suggested to be involved in tumor development and progression and to play an important role in cancer pathophysiology. However, the expression profile and functional significance of *N4bp2l1* in adipocyte development remain completely unknown.

In this study, we identified *N4bp2l1* as a novel USF1 target gene and found that its knockdown inhibited adipocyte differentiation in 3T3-L1 cells. This relationship between USF1 and *N4bp2l1* transcription in

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adipocytes could represent a new pathway in the regulation of adipogenesis, thereby providing targets for the therapeutic modulation of obesity.

2. Materials and methods

2.1. Cells and culture conditions

Human embryonic kidney 293T (HEK293T) and 3T3-L1 cells were cultured in low-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and 100 units of penicillin/streptomycin at 37 °C under 5% CO_2 atmosphere.

2.2. Adipocyte differentiation

3T3-L1 cells were cultured until confluence in high-glucose DMEM. Two days later, fresh high-glucose DMEM containing 5 μg/mL insulin, 1 μM dexamethasone (DEX), and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) introduced. After incubation for 48 h, the medium was changed to high-glucose DMEM containing 5 μg/mL insulin. The medium was changed every alternate day [8]. In experiments with adenovirus, 3T3-L1 cells were infected with adenovirus at day 2 before the induction of differentiation.

2.3. Animal experiments

For mice experiments, 8-week-old male C57BL/6J mice from Japan SLC were used. The mice were maintained on a standard chow diet (CE-2) consists of 50.3% carbohydrates, 25.4% protein and 4.4% fat (CLEA Japan). Animal experimental protocols were approved by the animal ethics committee of Jichi Medical University (permit number 17177), and were performed in accordance with the Use and Care of Experimental Animals Guidelines of the Jichi Medical University Guide for Laboratory Animals.

2.4. Western blotting

Total cell lysates were electrophoretically separated on denaturing polyacrylamide gels and transferred to polyvinylidene difluoride membranes. Proteins were detected with N4BP2L1 antibody (HPA038971, Merck) and β-actin antibody (G043, abm) as internal control.

2.5. Transfection and luciferase assays

We used the Dual-Luciferase Reporter Assay System (Promega) to determine promoter expression levels. Mouse *N4bp2l1* was generated with PCR using the mouse genomic DNA as a template. 3T3-L1 cells were co-transfected with each expression vector, mouse *N4bp2l1* promoter that drives firefly luciferase expression (pGL4.10 *mN4bp2l1*-Luc*)*, and *Renilla reniformis* luciferase vector (pGL4.74) for use as an internal control reporter. After transfection, the cells were incubated for 24 h at 37 °C under 5% CO_2 atmosphere and then lysed in 100 μL 1X Passive Lysis Buffer (Promega). Next, 10 μL lysate was used in the luciferase assay, and luminescence was detected using the Fluoroskan Ascent FL Microplate Luminometer (Thermo Fisher Scientific).

2.6. Electrophoretic mobility shift assay (EMSA)

Biotin-labeled probes are mixed with USF1-overexpressing nuclear protein extract (supershift lane is generated by adding the USF1 protein and USF1 antibody [sc-229; SantaCruz Biotech]) and allowed to incubate at room temperature for 20 min. DNA–protein bands were detected using the LightShift Chemiluminescent EMSA Kit (Thermo Fisher Scientific). The probes used for EMSA were N4bp2l1 Ebox2 and CAT CTATCACATGGCCGGAG.

2.7. Chromatin immunoprecipitation (ChIP) assay

ChIP assay was performed using the SimpleChIP Enzymatic Chromatin IP Kit (Cell Signaling Technology). Immunoprecipitation was performed using an antibody directed against USF1 and mouse IgG as the negative control. The associated DNA was then amplified with a primer pair: *N4bp2l1* promoter −86 Fwd 5′- CTCACAGGTAGAGACGC CCCCTC-3′ and *N4bp2l1* promoter +125 Rv 5′- CAGCCTCCCGAACGA TTCCAGAAAA-3′.

2.8. Oil Red O stain

The differentiated 3T3-L1 adipocytes were fixed for 10 min at 37 °C using 10% formaldehyde solution and then stained for 20 min at room temperature using freshly prepared 60% Oil Red O solution (Muto Pure Chemicals Co.). The cells were washed with PBS and rinsed with 60% isopropanol.

2.9. Adenoviral expression vectors

Adenoviruses were prepared and amplified with the ViraPower Adenoviral Expression System (Thermo Fisher Scientific), as previously described [22]. The shRNAs of *N4bp2l* and *LacZ* were cloned into BLOCK-iT U6 entry vector (Thermo Fisher Scientific). The shRNA sequence for *N4bp2l1* shRNA#1 was 5′-cacc GAATAACTATGAAGTTATA ttcaagaga TATAACTTCATAGTTATTC-3′ and for *N4bp2l1* shRNA#2 was cacc GAAAGAATTGGATTGAAAT ttcaagaga ATTTCAATCCAATTCT TTC. The pENTR vector inserts were transferred into the adenovirus vector pAd/PL-DEST using the Gateway System (Thermo Fisher Scientific). The recombinant adenoviruses were purified using the Adenovirus Purification Miniprep Kit (Cell Biolabs) according to the manufacturer's protocol.

2.10. Real-Time PCR (qPCR)

Total RNAs were isolated using acid guanidinium thiocyanate–-phenol reagent [23]. cDNA was synthesized using the Verso cDNA Kit (Thermo Fisher Scientific) with random hexamer primers. qPCR assays were performed using the ViiA7 Real-Time PCR System and KAPA SYBER FAST ROX Low qPCR Kit (Kapa Biosystems). The relative gene expression levels were quantified with qPCR, followed by normalization to the internal control gene ribosomal protein lateral stalk subunit P0 (*Rplp0*). The primers used for this analysis were N4bp2l1 Fwd, 5′-TGGAACCAGAGAAGAGCAAGGA-3′ and N4bp2l1 Rv, 5′-AGTGTCG GGTTCCCGGAATATAA-3′; C/ebpa Fwd, 5′-GCCATGCCGGGAGAACT CTA-3′ and C/ebpa Rv, 5′- GGGCTCTGGAGGTGACTGCT-3′; C/ebpb Fwd, 5′-GAGCCGCGACAAGGCCAAGA-3′ and C/ebpb Rv, 5′-GCTCGTT CTCCGCCGTCAGC-3′; Pparg Fwd, 5′- TTCCACTATGGAGTTCATGCT TGT-3′ and Pparg Rv, 5′- TCCGGCAGTTAAGATCACACCTA-3′; Rplp0 Fwd, 5′-ATGCAGCAGATCCGCATGT-3′ and Rplp0 Rv, 5′-TTGCGCATC ATGGTGTTCTT-3′.

2.11. Statistical analysis

Experimental studies were performed in triplicates or in greater numbers of sets. Statistical significance was tested using the unpaired two-tailed Student's *t*-test or one-way ANOVA with Bonferroni test for multiple comparisons. Data were expressed as mean ± SEM. Statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. N4bp2l1 expression during 3T3-L1 adipocyte differentiation

To identify novel genes that regulate adipocyte development, we first searched the expression profiles during *in vitro* adipocyte

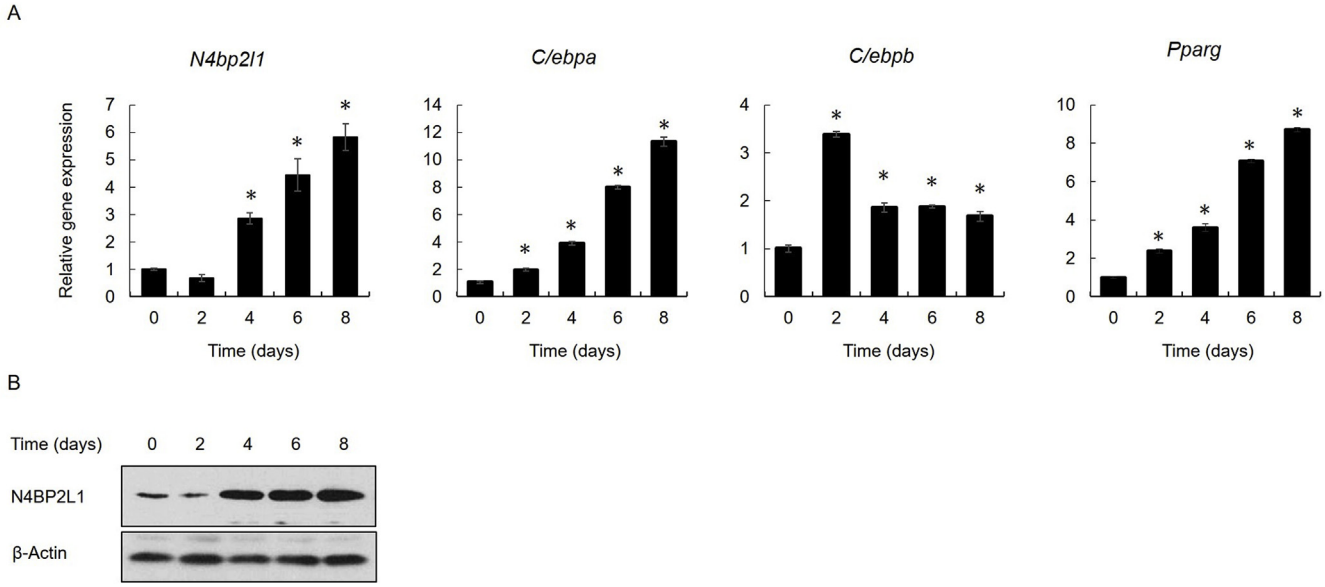

Fig. 1. *N4bp2l1* mRNA and N4BP2l1 protein expression during 3T3-L1 cell differentiation. Total RNA and whole cell lysates were extracted from 3T3-L1 cells on days 0, 2, 4, 6, and 8 after the induction of differentiation by treatment with an adipogenic cocktail. (A) The expression levels of *N4bp2l1*, *C/ebpa*, *C/ebpb*, and *Pparg* were measured using qPCR, followed by normalization to the internal control gene *Rplp0*; $n = 3$ per group, $^{*}p < 0.01$ vs. day 0. (B) Western blot showing N4BP2l1 protein levels.

differentiation. To follow this approach, we used a publicly available microarray data from Gene Expression Omnibus (GSE20696) [24]; preadipocyte 3T3-L1 cells were compared at day0 and at day 7 after differentiation. Interestingly, these data indicated that *N4bp2l1* was dramatically increased by 23.9-fold at day 7 compared with that at day 0 (GSE20696). Next, to confirm the evidence for *N4bp2l1* expression profiling of 3T3-L1 adipogenesis, we analyzed the expression of *N4bp2l1* in 3T3-L1 cell differentiation using qPCR. A cocktail mix comprising DEX, IBMX, and insulin was used to induce differentiation in 3T3-L1 cells. Total RNA was extracted from the cells on days 0, 2, 4, 6, and 8 after adipocyte differentiation was induced. *N4bp2l1* expression increased by three-fold between days 0 and 4, and during 3T3-L1 cell differentiation, *N4bp2l1* expression subsequently increased by six-fold on day 8 compared with that on day 0 (Fig. 1A). N4BP2l1 protein levels markedly increased between days 0 and 4 following adipocyte differentiation (Fig. 1B). These results suggested that N4BP2L1 was constitutively expressed in preadipocyte 3T3-L1 cells at day 0 and remarkably increased after induction of adipocyte differentiation (Fig. 1B). Fig. 1A shows the expression of *C/ebpa* and *Pparg*, the transcription factors induced during adipocyte differentiation. In addition, the expression of *C/ebpb*, the key early regulator of adipogenesis, was induced. The increase in *N4bp2l1* expression level after adipocyte differentiation in our results was not as high as that reported in the GEO database. However, in tissue distribution of mice, *N4bp2l1* was highly expressed in brain and adipose tissue (Supplementary Fig. 1). Because the brain and adipose tissue are lipid rich, *N4bp2l1* may be related to lipid metabolism. In addition, from the Type 2 Diabetes Knowledge Portal (http://www.type2diabetesgenetics.org/, last accessed on December 18, 2018), we detected nominally significant associations between the variant allele of *N4BP2L1* rs497255 and BMI in the GIANT UK Biobank GWAS ($p = 1.6 \times 10^{-15}$). These results suggest that *N4bp2l1* expression is associated with the link between lipid accumulation and BMI and plays an important role in lipid-rich organ.

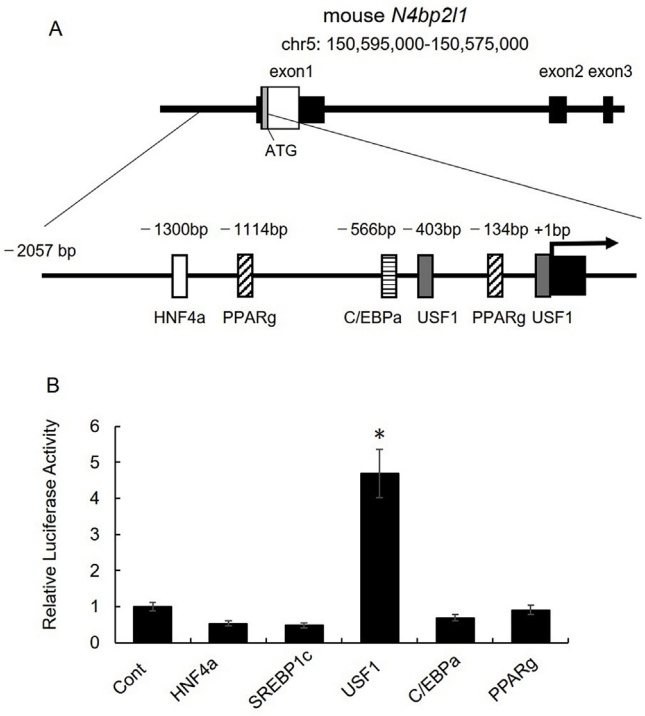

Fig. 2. Activation of *N4bp2l1* promoter by USF1. (A) A schematic of the mouse *N4bp2l1* promoter with the potential binding sites of adipogenesis- and lipid metabolism-related transcription factors. (B) The mouse *N4bp2l1* promoter region (2057 bp) was fused to a luciferase reporter gene (pGL4.10 *mN4bp2l1* − 2057-Luc). The 3T3-L1 cells were cotransfected with pGL4.10 *mN4bp2l1* − 2057-Luc as a reporter gene, *Renilla reniformis* luciferase vector (pGL4.74) served as an internal control reporter, and the indicated expression plasmids or control vector (pcDNA3.1). $n = 3$ per group, $^{*}p < 0.01$ vs. control.

3.2. Regulation of N4bp2l1 promoter by USF1

Fig. 2A shows the structure of mouse *N4bp2l1*. We used the LASAGNA-Search 2.0 to identify potential binding sites of adipogenesis- and lipid metabolism-related transcription factors in the *N4bp2l1* promoter [25]. We recognized putative transcription factor-binding sites for hepatocyte nuclear factor 4 alpha (HNF4a), PPARg, C/EBPa, and USF1 (Fig. 2A). Hepatocyte nuclear factor-4 (HNF4), in addition to inducing numerous genes involved in lipid metabolism, binds to direct

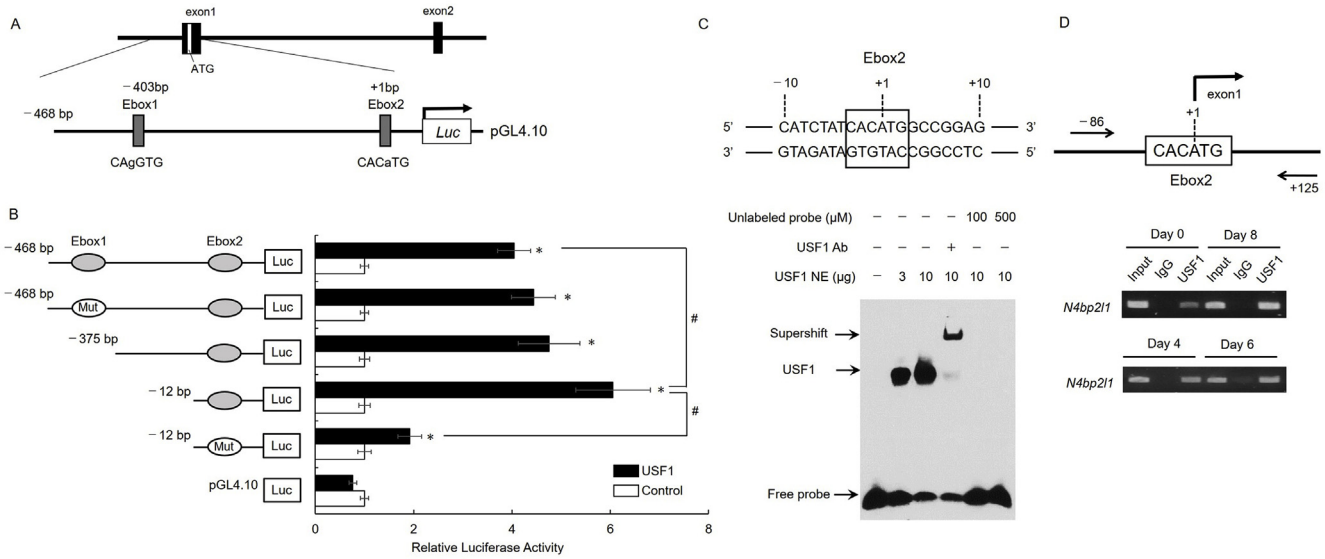

Fig. 3. *N4bp2l1* promoter is a direct target of USF1. (A) A schematic of the potential Ebox element of the mouse *N4bp2l1* promoter. (B) Luciferase assays of the reporter gene including the mouse *N4bp2l1* promoter region (−468 bp) and its various deletion constructs in the presence or absence of the USF1 expression vector in 3T3-L1 cells. $n = 3$ per group, $^{*}p < 0.01$ vs. control and $^{\#}p < 0.01$ vs. −12 bp *N4bp2l1* promoter with USF1. (C) Upper panel: Ebox2 of the mouse *N4bp2l1*. Bottom panel: EMSA for USF1 binding to Ebox2 of the *N4bp2l1* promoter. Nuclear extract (NE) of USF1 protein was incubated with biotin-labeled Ebox2 of the *N4bp2l1* promoter probe in the presence or absence of unlabeled probe and USF1 antibody. The competition was induced using an unlabeled probe as a competitor at 100- and 500-fold molar excesses. (D) ChIP assay for USF1 binding to Ebox2 of the *N4bp2l1* promoter using 3T3-L1 preadipocytes (day 0) or adipocytes after 3T3-L1 differentiation (day 4, 6 and 8). The extracted genomic DNA was subjected to immunoprecipitation performed with an antibody against USF1 and with IgG as the negative control. For comparison, amplification derived from unprecipitated chromatin is also shown (input). $n = 3$ per group, $^{*}p < 0.01$ vs. day 0.

repeat motifs of the AGGTCA sequence that are separated by one nucleotide (DR1) and regulates gene expression [26]. PPARg also binds to the DR1 element as a heterodimer with the retinoid X receptor family [27]. C/EBP family members recognize the palindromic DNA sequence ATTGCGCAAT [28]. USF1 specifically binds to the Ebox sequence CACGTG [9]. To determine whether these transcription factors activate the *N4bp2l1* promoter, the 2-kb 5′ region upstream of the mouse *N4bp2l1* promoter was cloned into a luciferase vector and subjected to a luciferase reporter assay using 3T3-L1 cells. The *N4bp2l1* promoter was significantly activated by the coexpression of USF1 (Fig. 2B), which suggests that *N4bp2l1* is a novel USF1 target gene.

3.3. Requirement of Ebox regions for USF1 activation of N4bp2l1 promoter

USF1 binds to the Ebox motif CACGTG in the promoter of lipid metabolism-related genes, such as apolipoproteins [10–13], hormone-sensitive lipase [17], ATP-binding cassette transporter-1 [15], and fatty acid synthase [9,16]. We identified 2 predicted Ebox sites in the 0.5-kb 5′ region upstream of the mouse *N4bp2l1* (Fig. 3A). The region responsible for USF1 activation was located within −468 bp, indicating that the Ebox1 sequence at −403 bp (CAgGTG) and the Ebox2 sequence at +1 (CACaTG) could be the USF1-binding sites (Fig. 3A). To confirm whether the Ebox motif of the *N4bp2l1* promoter was the correct USF1-binding site, various deletion and mutation constructs (as indicated in Fig. 3B) were prepared and tested for the basal and USF1-induced activities. The *N4bp2l1* promoter including Ebox1 and Ebox2 was significantly activated by USF1 coexpression (Fig. 3B). Although there is no Ebox1 site in Ebox1 mutation and Ebox1 deletion (−375 bp) luciferase vectors, the expression was significantly promoted by USF1 coexpression (Fig. 3B). Ebox2 in the −12 bp *N4bp2l1* promoter resulted in a significant increase in transcript abundance. Notably, the activation of the −12 bp *N4bp2l1* promoter was significantly increased compared with the −468 bp promoter (Fig. 3B), suggesting that other regions (from −468 to − 12 bp) of the *N4bp2l1* promoter have suppressing domain responsible for *N4bp2l1* expression. In addition, activation of the −12 bp mutant *N4bp2l1* promoter significantly increased by USF1 compared with that by control. This result indicated that USF1 might slightly bind to the −12bp mutant *N4bp2l1* promoter. However, Ebox2 mutation in the −12 bp *N4bp2l1* promoter caused a significant decline in USF1 activation compared with the −12 bp *N4bp2l1* promoter, which suggests that Ebox2 is a crucial USF1-binding site for *N4bp2l1* transcription (Fig. 3B).

EMSA was performed to confirm the direct binding of USF1 to the Ebox2 sequence, which indicated that USF1 binds to the Ebox2 oligonucleotide and USF1 antibody binds to the USF1/Ebox2 oligonucleotide complex, resulting in band shifting (supershift). The protein binding specificity of the Ebox2 probe was demonstrated by competition with 100- and 500-fold molar excesses of the unlabeled Ebox2 probe (Fig. 3C).

To elucidate whether USF1 physically binds to the endogenous mouse *N4bp2l1* promoter, we performed the ChIP assay using pre-adipocytes and mature adipocytes of 3T3-L1. The results of ChIP assay of genomic DNA from before differentiation at day 0 (preadipocytes) or after differentiation at day 4, 6, and 8 (mature adipocytes) of 3T3-L1 confirmed the direct binding of USF1 to Ebox2 *in vitro*; moreover, signal intensity detected at day 0 was increased at day 8 (Fig. 3D); this finding is consistent with *N4bp2l1* expression during 3T3-L1 differentiation (Fig. 1A). Furthermore, during 3T3-L1 cell differentiation, *Usf1* expression decreased by 45% between days 0 and 2, and then increased in a time-dependent manner (Supplementary Fig. 2A). *N4bp2l1* expression also decreased by 33% between days 0 and 2, and then increased at day 4 following adipocyte differentiation (Fig. 1A). Although *Usf1* mRNA expression levels were not consistent with USF1 protein levels, increase expression levels of USF1 protein were consistent with N4BP2L1 protein levels during 3T3-L1 differentiation (Fig. 1B and Supplementary Fig. 2B). These results suggested that increased USF1 following lipid accumulation in adipocytes binds to the *N4bp2l1* promoter and upregulates *N4bp2l1* transcripts. Our results indicate that USF1 activates *N4bp2l1* transcription by specifically binding to the Ebox sequence in the *N4bp2l1* promoter and that *N4bp2l1* is a novel USF1 target gene involved in lipid metabolism.

Triglyceride accumulation and adipocyte differentiation in 3T3-L1

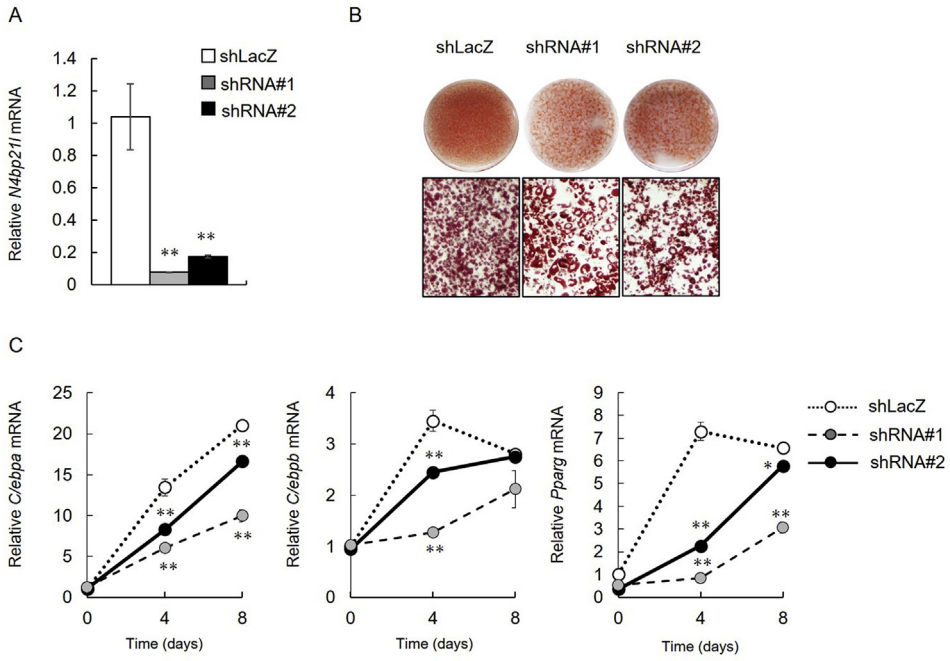

Fig. 4. The effects of *N4bp2l1* knockdown on 3T3-L1 cellular differentiation. (A) The expression of *N4bp2l1* mRNA in *N4bp2l1* knockdown 3T3-L1 cells at day 8. *N4bp2l1*-knockdown adenovirus particles were used with either of the 2 independent *N4bp2l1* shRNA constructs (shRNA#1 and shRNA#2); $n = 3$ per group, $^{*}p < 0.01$ vs. shLacZ. (B) Triglyceride accumulation in 3T3-L1 cells on day 8, visualized using Oil Red O staining. (C) The relative mRNA levels in 3T3-L1 cells expressing each shRNA on days 0, 4, and 8 after inducing differentiation. $n = 3$ per group, $^{*}p < 0.01$ vs. shLacZ. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

cells were inhibited by *N4bp2l1* knockdown.

To investigate *N4bp2l1*'s role in adipocyte differentiation, we studied *N4bp2l1* knockdown effects on 3T3-L1 differentiation. Adenoviral infection of either of the 2 independent *N4bp2l1* shRNA constructs decreased *N4bp2l1* mRNA expression levels by approximately 80%–90% compared with that of the shLacZ control (Fig. 4A). On day 8 after differentiation induction, the shLacZ control cells displayed abundant lipid droplets (observed with Oil Red O staining) (Fig. 4B) and significantly increased expression of *C/ebpa*, *C/ebpb*, and *Pparg* (Fig. 4C). In contrast, the levels of lipid accumulation and adipocyte differentiation markers (*C/ebpa*, *C/ebpb*, and *Pparg*) significantly decreased in both *N4bp2l1*-knockdown cells. These results suggest that *N4bp2l1* plays an important role in triglyceride accumulation and adipocyte differentiation.

The study results demonstrate that USF1 regulates *N4bp2l1* mRNA expression and that they may be associated with lipid metabolism in the early and/or late stage of 3T3-L1 adipocyte differentiation. Elucidation of the physiological function of N4BP2l1 function(s) may contribute to a new therapeutic target for obesity, although further research is needed.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2019.100676.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrep.2019.100676

References

[1] E.J. Gallagher, D. LeRoith, Obesity and Diabetes: the increased risk of cancer and cancer-related mortality, Physiol. Rev. 95 (2015) 727–748.
[2] E. Hui, A. Xu, H. Bo Yang, K.S. Lam, Obesity as the common soil of non-alcoholic fatty liver disease and diabetes: role of adipokines, J. Diabetes Invest. 4 (2013) 413–425.
[3] I. Vucenik, J.P. Stains, Obesity and cancer risk: evidence, mechanisms, and recommendations, Ann. N. Y. Acad. Sci. 1271 (2012) 37–43.
[4] L. Luo, M. Liu, Adipose tissue in control of metabolism, J. Endocrinol. 231 (2016) R77–r99.
[5] D. Moseti, A. Regassa, W.K. Kim, Molecular regulation of adipogenesis and potential anti-adipogenic bioactive molecules, Int. J. Mol. Sci. 17 (2016).
[6] F.J. Ruiz-Ojeda, A.I. Ruperez, C. Gomez-Llorente, A. Gil, C.M. Aguilera, Cell models and their application for studying adipogenic differentiation in relation to obesity: a Review, Int. J. Mol. Sci. 17 (2016).
[7] H.J. Zhu, H.H. Ding, J.Y. Deng, H. Pan, L.J. Wang, N.S. Li, X.Q. Wang, Y.F. Shi, F.Y. Gong, Inhibition of preadipocyte differentiation and adipogenesis by zinc-alpha2-glycoprotein treatment in 3T3-L1 cells, J. Diabetes Invest. 4 (2013) 252–260.
[8] K. Watanabe, K. Yoshida, S. Iwamoto, Kbtbd11 gene expression in adipose tissue increases in response to feeding and affects adipocyte differentiation, J. Diabetes Invest. 10 (2019) 925–932.
[9] V.S. Vallet, M. Casado, A.A. Henrion, D. Bucchini, M. Raymondjean, A. Kahn, S. Vaulont, Differential roles of upstream stimulatory factors 1 and 2 in the transcriptional response of liver genes to glucose, J. Biol. Chem. 273 (1998) 20175–20179.
[10] A. Ribeiro, D. Pastier, D. Kardassis, J. Chambaz, P. Cardot, Cooperative binding of upstream stimulatory factor and hepatic nuclear factor 4 drives the transcription of the human apolipoprotein A-II gene, J. Biol. Chem. 274 (1999) 1216–1225.
[11] M. Nowak, A. Helleboid-Chapman, H. Jakel, G. Martin, D. Duran-Sandoval, B. Staels, E.M. Rubin, L.A. Pennacchio, M.R. Taskinen, J. Fruchart-Najib, J.C. Fruchart, Insulin-mediated down-regulation of apolipoprotein A5 gene expression through the phosphatidylinositol 3-kinase pathway: role of upstream stimulatory factor, Mol. Cell. Biol. 25 (2005) 1537–1548.
[12] D. Pastier, J.M. Lacorte, J. Chambaz, P. Cardot, A. Ribeiro, Two initiator-like elements are required for the combined activation of the human apolipoprotein C-III promoter by upstream stimulatory factor and hepatic nuclear factor-4, J. Biol. Chem. 277 (2002) 15199–15206.
[13] J. Naukkarinen, M. Gentile, A. Soro-Paavonen, J. Saarela, H.A. Koistinen, P. Pajukanta, M.R. Taskinen, L. Peltonen, USF1 and dyslipidemias: converging evidence for a functional intronic variant, Hum. Mol. Genet. 14 (2005) 2595–2605.
[14] G.J. Botma, A.J. Verhoeven, H. Jansen, Hepatic lipase promoter activity is reduced by the C-480T and G-216A substitutions present in the common LIPC gene variant, and is increased by Upstream Stimulatory Factor, Atherosclerosis 154 (2001) 625–632.
[15] X.P. Yang, L.A. Freeman, C.L. Knapper, M.J. Amar, A. Remaley, H.B. Brewer Jr., S. Santamarina-Fojo, The E-box motif in the proximal ABCA1 promoter mediates transcriptional repression of the ABCA1 gene, J. Lipid Res. 43 (2002) 297–306.
[16] J.C. Lee, A.J. Lusis, P. Pajukanta, Familial combined hyperlipidemia: upstream transcription factor 1 and beyond, Curr. Opin. Lipidol. 17 (2006) 101–109.

[17] P. Pajukanta, I. Nuotio, J.D. Terwilliger, K.V. Porkka, K. Ylitalo, J. Pihlajamaki, A.J. Suomalainen, A.C. Syvanen, T. Lehtimaki, J.S. Viikari, M. Laakso, M.R. Taskinen, C. Ehnholm, L. Peltonen, Linkage of familial combined hyperlipidaemia to chromosome 1q21-q23, Nat. Genet. 18 (1998) 369–373.
[18] K. Kristiansson, E. Ilveskoski, T. Lehtimaki, L. Peltonen, M. Perola, P.J. Karhunen, Association analysis of allelic variants of USF1 in coronary atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 28 (2008) 983–989.
[19] W. Putt, J. Palmen, V. Nicaud, D.A. Tregouet, N. Tahri-Daizadeh, D.M. Flavell, S.E. Humphries, P.J. Talmud, Variation in USF1 shows haplotype effects, gene : gene and gene : environment associations with glucose and lipid parameters in the European Atherosclerosis Research Study II, Hum. Mol. Genet. 13 (2004) 1587–1597.
[20] T. Sasahira, T. Kirita, Hallmarks of cancer-related newly prognostic factors of oral squamous cell carcinoma, Int. J. Mol. Sci. 19 (2018).
[21] T. Sasahira, M. Kurihara, Y. Nishiguchi, R. Fujiwara, T. Kirita, H. Kuniyasu, NEDD 4 binding protein 2-like 1 promotes cancer cell invasion in oral squamous cell carcinoma, Virchows Arch. : Int. J. Pathol. 469 (2016) 163–172.
[22] K. Watanabe, E. Watson, M.L. Cremona, E.J. Millings, J.H. Lefkowitch, S.G. Fischer, C.A. LeDuc, R.L. Leibel, ILDR2: an endoplasmic reticulum resident molecule mediating hepatic lipid homeostasis, PLoS One 8 (2013) e67234.
[23] K. Watanabe, K. Nakayama, S. Ohta, K. Tago, S. Boonvisut, E.J. Millings, S.G. Fischer, C.A. LeDuc, R.L. Leibel, S. Iwamoto, ZNF70, a novel ILDR2-interacting protein, contributes to the regulation of HES1 gene expression, Biochem. Biophys. Res. Commun. 477 (2016) 712–716.
[24] T.S. Mikkelsen, Z. Xu, X. Zhang, L. Wang, J.M. Gimble, E.S. Lander, E.D. Rosen, Comparative epigenomic analysis of murine and human adipogenesis, Cell 143 (2010) 156–169.
[25] C. Lee, C.H. Huang, LASAGNA-Search 2.0: integrated transcription factor binding site search and visualization in a browser, Bioinformatics 30 (2014) 1923–1925.
[26] S. Harish, T. Khanam, S. Mani, P. Rangarajan, Transcriptional activation by hepatocyte nuclear factor-4 in a cell-free system derived from rat liver nuclei, Nucleic Acids Res. 29 (2001) 1047–1053.
[27] M.I. Lefterova, A.K. Haakonsson, M.A. Lazar, S. Mandrup, PPARgamma and the global map of adipogenesis and beyond, Trends Endocrinol. Metab.: TEM (Trends Endocrinol. Metab.) 25 (2014) 293–302.
[28] D.M. Cohen, H.W. Lim, K.J. Won, D.J. Steger, Shared nucleotide flanks confer transcriptional competency to bZip core motifs, Nucleic Acids Res. 46 (2018) 8371–8384.