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# Hyperproduction of IL-6 caused by aberrant TDP-43 overexpression in high-fat diet-induced obese mice

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ABSTRACT

**Inclusion of Tat-activating regulatory DNA-binding protein-43 (TDP-43) due to hyperphosphorylation or hyperubiquitination is a cause of neurodegenerative disease. Cellular TDP-43 expression is tightly controlled through a negative feedback loop involving its mRNA. Recently, we reported that the TDP-43-mediated sub-nuclear body is an essential site of interleukin-6 (IL-6) pre-mRNA processing. Here we show that mice fed on a high-fat diet exhibit increased TDP-43 expression in the liver and adipose tissue with a prominent increase in IL-6. TDP-43 depletion *in vivo* reduces IL-6 production in the liver. Overexpression or depletion of TDP-43 in pre-adipose and adipose cells causes reciprocal alteration of IL-6 expression and RNA processing. Our findings provide evidence for a link between homeostasis of TDP-43 expression and the risk of developing obesity.**


## 1. Introduction

Tat-activating regulatory DNA-binding protein-43 (TDP-43) is structurally related to the family of heterogeneous nuclear ribonucleoproteins and is involved in RNA processing [1]. This protein was originally identified as a transcription factor that binds to the human immunodeficiency virus transactivation response region to repress transactivation response-mediated transcription [2]. Structurally, TDP-43 contains two classical RNA recognition motif (RRM) domains flanked on either side by N-terminal and glycine-rich C-terminal domains. The RRM1 domain is necessary for nucleic-acid binding, but the RRM2 domain does not play a significant role in RNA binding. The C-terminal glycine-rich domain is important for protein–protein interactions [3,4]. TDP-43 is a major component of the cytoplasmic inclusions that characterize the central nervous system pathology involved in frontotemporal lobar degeneration and amyotrophic lateral sclerosis [5,6]. In normal cells, TDP-43 is localized mainly in the nucleus, while in affected cells, ubiquitinated TDP-43 aggregates are mislocalized in the cytoplasm due to mutations within the TDP-43 gene [6]. Aberrantly truncated and phosphorylated TDP-43 is also present in the intracellular ubiquitinated cytoplasmic inclusions [6,7].

Interleukin-6 (IL-6) is a soluble mediator with pleiotropic effects on inflammation, immune response, and hematopoiesis; therefore, dysregulation of IL-6 expression results in pathological effects related to chronic inflammation and autoimmunity. In particular, obese patients exhibit sustained liver inflammation and increased plasma IL-6 levels [8]; thus, IL-6 is a possible molecular marker for obesity-induced inflammation. We previously reported that TDP-43-containing sub-nuclear bodies are specific cytokine-splicing compartments important for efficient RNA production during activation and modulation of the immune response [9]. In addition, knockdown of TDP-43 disrupts the regulation of IL-6 expression and RNA processing. Although TDP-43 controls its own homeostasis by downregulating transcript levels [10], a possible correlation between aberrant TDP-43 overexpression and IL-6 hyperproduction in obesity may exist.

Here, we demonstrate that TDP-43 expression is highly elevated in the liver and adipose tissue in concert with increased IL-6 levels in high-fat diet-fed mice. Specifically, overexpression of TDP-43 significantly enhances IL-6 levels in pre-adipocytes, adipocytes, and macrophages. The effect of TDP-43 overexpression on IL-6 production in these cells, along with its positive correlation with obesity, is concordant with TDP-43's role as a critical regulator of IL-6 RNA processing. Indeed, depletion of TDP-43 *in vivo* leads to a

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dramatic reduction in IL-6 production and RNA processing in the liver of mice. Taken together, these results suggest that TDP-43-dependent regulation of IL-6 expression underlies the pathological link between TDP-43 and obesity.

## 2. Materials and methods

### 2.1. DNA constructs

Mouse TDP-43 construct was fused at the N-terminus to HA and cloned into the retroviral pLHCX (Hygro) vector (Clontech, Mountain View, CA). The shRNA sequence against TDP-43 was annealed and subcloned into the pSUPER retroviral vector (Oligoengine, Seattle, WA) using the following primers: 5′-GATCC GCGATGAACCCATTGAAATACTCGAGTATTTCAATGGGTTCATCGTTTT TTGGAAA-3′ and 5′-AGCTTTTCCAAAAAACGATGAACCCATTGAAATA CTCGAGTATTTCAATGGGTTCATCGCG-3′. The shRNA sequence against GFP, which was used as a negative control, was subcloned using the following primers: 5′-GATCCGCAAGCTGACCCTGAAGTTC CTCGAGGAACTTCAGGGTCAGCTTGCTTTTTTGGAAA-3′ and 5′-AGCT TTTCCAAAAAAGCAAGCTGACCCTGAAGTTCCTCGAGGAACTTCAGGG TCAGCTTGCGG-3′. All constructs were verified by sequencing.

### 2.2. Antibodies

Antibodies against TDP-43 (#A303-223A), β-actin (#A5316), tubulin (#G094) and GAPDH (#sc-32233) were obtained from Bethyl Laboratories (Montgomery, TX), Sigma (St. Louis, MO), ABM Inc. (Richmond, Canada) and Santa Cruz Biotechnology (Santa Cruz, CA) respectively.

### 2.3. Adipocyte differentiation

3T3-L1 cells were cultured in DMEM with bovine calf serum (Life Technologies, Carlsbad, CA) and 1% antibiotics (HyClone, Logan, UT). 3T3-L1 cells were induced to differentiate into adipocytes. Briefly, after 2 days of confluence (day 0), cells were stimulated with DMEM containing 10% fetal bovine serum (FBS) and MDI (0.5 mM isobutylmethylxanthine, 1 μM dexamethasone, and 10 μg/ml insulin; Sigma). After 2 days, the medium was changed to DMEM containing 10% FBS and 10 μg/ml insulin. The medium was then changed to DMEM containing 10% FBS and changed every 2 days. Differentiation was achieved by day 10–12.

### 2.4. Animals and diets

Five-week-old male C57BL/6N mice were obtained from Orient Bio (Gyeonggi-do, South Korea). All animals were housed in specific pathogen-free conditions at 21 ± 2.0 °C temperature with 50 ± 5% relative humidity and a 12 h-light/12 h-dark cycle. Before the diet intervention was started, all animals were fed standard chow. At the beginning of the study, mice were divided into 2 groups: (1) control group fed the normal diet (ND, $n = 8$) and (2) a group fed the high-fat diet (HFD, $n = 8$). Mice were provided food and water *ad libitum*. The body weight and food intake were monitored throughout the study. At 12 weeks after the initiation of the study, 8 animals from each group were sacrificed. Mouse serum was collected for ELISA assay. Tissues were snap-frozen immediately in liquid nitrogen and stored at −80 °C until further processing.

### 2.5. Retroviral transduction

HEK 293T cells were transfected with plasmids encoding VSV-G and Gag-Pol, as well as either HA-TDP-43, shRNA for TDP-43, or negative control shRNA for GFP. Approximately 36–48 h post-transfection, media containing viral particles were collected, filtered through a 0.45-μm membrane, and incubated with RAW macrophages or pre-adipocytes for 24 h. Cells were selected with hygromycin or puromycin.

### 2.6. ELISA

Mouse serum or tissue culture supernatant was collected, and IL-6 levels were analyzed by ELISA according to the manufacturer's recommendations (BD Biosciences, San Jose, CA).

### 2.7. Immunofluorescence assay

For immunofluorescent staining, cells were fixed in 3.7% formaldehyde and permeabilized with 0.2% Triton X-100 prior to incubation with the appropriate primary antibody. Bound antibody was visualized with an Alexa Fluor 488-conjugated secondary antibody (Life Technologies). DAPI (4′,6-diamidino-2-phenylindole; Sigma) was used as a nuclear counterstain.

### 2.8. Real-time PCR and RT-PCR assays

Total cellular RNA was prepared using an RNA prep kit (GeneAll, Seoul, South Korea). Then, 500 ng total RNA was reverse transcribed for 1 h with random hexamers at 42 °C using Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (Enzynomics, Daejeon, South Korea). PCR was performed using the following conditions: 30 cycles of 15 s at 94 °C, 15 s at the experimentally determined optimal annealing temperature (56–58 °C), and 60 s per 1 kb at 72 °C (15–40 s). PCR products were visualized on ethidium bromide-stained gels. GAPDH was used for normalization. Real-time PCR was performed using TOPreal qPCR premix (SYBR Green, Enzymonics) and Applied Biosystems 7300 Real-Time PCR System (Life Technologies). Results were

**Table 1**
PCR primer sequences and sizes of pre-mRNA or mRNA.

| Gene | Size of pre-mRNA | Size of mRNA | Forward Primer (Target region)<br>Reverse Primer (Target region) |
|---|---|---|---|
| IL-6 (RT-PCR) | 332 | 167 | 5′-ACCGCTATGAAGTTCCTCTCTGCA-3′ (Exon 1)<br>5′-AAGCCTCCGACTTGTGAAGTGGT-3′ (Exon 2) |
| IL-6 (qRT-PCR) | 1338 | 112 | 5′-GTCCTTCAGAGAGATACAGAAACT-3′ (Exon 4)<br>5′-AGCTTATCTGTTAGGAGAGCATTG-3′ (Exon 5) |
| TNF-α (RT-PCR) | 634 | 340 | 5′-ACAAGCCTGTAGCCCACGTC-3′ (Exon 3)<br>5′-AAGACTCCTCCCAGGTATATGG-3′ (Exon 4) |
| TDP-43 (RT-PCR/qRT-PCR) | 3417 | 405 | 5′-ATGTCTGAATATATTCGGGTAACAGAAG-3′ (Exon1)<br>5′-GACCTGAACCATAAGAACCTCTCC-3′ (Exon2) |
| GAPDH (RT-PCR) | 594 | 376 | 5′-CCTGGCCAAGGTCATCCATG-3′ (Exon 3)<br>5′-GCAGGAGACAACCTGGTCCT-3′ (Exon 5) |
| GAPDH (qRT-PCR) | 1931 | 97 | 5′-CCTGGCCAAGGTCATCCATG-3′ (Exon 1)<br>5′-GCAGGAGACAACCTGGTCCT-3′ (Exon 2) |

normalized to expression of the gene encoding GAPDH and were quantified by the change-in-threshold method (ΔΔCT). All primer sequences are listed in Table 1.

### 2.9. In vivo RNAi experiment

TDP-43 expression was knocked down in live mice with TDP-43-specific siRNA using Invivofectamine (Invitrogen). The siRNA-Invivofectamine complex was prepared according to the manufacturer's protocol. Briefly, siRNA (3 mg/ml) was mixed with complexation buffer and Invivofectamine, incubated for 30 min at 50 °C, and dialyzed at room temperature for 2 h in phosphate-buffered saline (PBS; pH 7.4). Dialyzed siRNA-Invivofectamine complex (7 mg/kg) was injected via tail veins of female C57BL/6 (5 or 6 weeks old). Two days later, mice were treated intraperitoneally with LPS (5 mg/kg). After 9 h treatment, mice were euthanized and liver tissues were collected for qRT-PCR or RT-PCR.

### 2.10. Immunoblot analysis

Cell lysates were separated by SDS–PAGE, transferred onto nitrocellulose membranes, blocked with 5% skim milk in PBS containing 0.1% Tween-20 (Sigma) for 2 h, and then probed with the appropriate antibody for 4 h at RT. Membranes were washed three times with PBS containing 0.1% Tween-20 and incubated with horseradish peroxidase-conjugated streptavidin for 1 h. Bands were visualized using an ECL detection reagent (AbClon, Seoul, South Korea).

### 2.11. Ethics statement

All animal experiments were performed in accordance with the Korean Food and Drug Administration (KFDA) guidelines. Protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Yonsei Laboratory Animal Research Center (YLARC) at Yonsei University (Permit Number: 2007-0001).

### 2.12. Statistical analysis

All experiments were repeated at least three times with consistent results. Data are presented as mean and S.D. or S.E.M. (as noted in figure legends). Statistical differences between two means were evaluated with a two-tailed unpaired Student's *t*-test. Differences with *P*-values less than 0.05 were considered significant. No samples were excluded from the analysis. The data had a normal distribution, and the variance was similar between the groups being compared. No statistical method was used to predetermine sample sizes. Sample size was based on previous experience with experimental variability. The experiments were not randomized. The investigators were not blinded to allocation during experiments or outcome assessment.

## 3. Results

### 3.1. High expression levels of TDP-43 in HFD-induced obesity

As previously noted, the TDP-43-containing sub-nuclear body is involved in IL-6 RNA processing [9]. Since TDP-43 levels are under tight control via a negative feedback loop at the mRNA level [10], TDP-43 overexpression may affect IL-6 overproduction, which is strongly related with inflammatory disease phenotypes such as obesity. To investigate the relationship between aberrant TDP-43 overexpression and obesity, we first analyzed the body weight and IL-6 levels in C57BL/6N mice fed a HFD compared to those animals fed a ND. The body weights of HFD mice continued to increase over the course of experimental feeding, and the difference in weight between the two groups was approximately 45% at 12 weeks (Fig. 1A, left). The HFD mice also exhibited significantly higher levels of IL-6 than ND mice (Fig. 1A, right). To determine if IL-6 levels correlate with TDP-43 expression during obesity, we assessed TDP-43 expression in the liver and adipose tissue (epididymal and subcutaneous fat) of HFD mice. Interestingly, TDP-43 mRNA and protein levels were significantly increased in the liver and adipose tissue of HFD mice compared to ND mice (Fig. 1B and C). These results indicate that increased TDP-43 expression levels are positively linked to elevated IL-6 expression and obesity.

### 3.2. TDP-43 expression directly impacts IL-6 production in pre-adipocytes, adipocytes, and macrophages

Adipose tissue is a main source of IL-6 during obesity [11,12], so to further examine the role of TDP-43 in obesity, we generated pre-adipose and adipose cell lines that stably overexpress HA-TDP-43 and then evaluated their IL-6 production. Interestingly, we found that overexpression of TDP-43 significantly increased IL-6 levels in culture supernatants (Fig. 2A). To confirm this association, we analyzed the effect of TDP-43 depletion on IL-6 levels in both cell lines. In contrast to results regarding TDP-43 overexpression, TDP-43-depleted pre-adipose and adipose cells showed considerably reduced IL-6 production (Fig. 2B). Examination of TDP-43's effect on IL-6 RNA processing revealed that IL-6 mRNA stability increased in TDP-43-overexpressed pre-adipocytes (Fig. 2C) and that levels of IL-6 mRNA were reduced in TDP-43-depleted pre-adipocytes and adipocytes (Fig. 2D, arrowheads). However, levels of TNF-α mRNA were unaffected (Fig. 2D), as TDP-43 binds RNAs in a sequence-specific manner [9]. Moreover, knockdown of TDP-43 induced the accumulation of unspliced IL-6 pre-mRNA, indicating significant impairment of IL-6 RNA processing (Fig. 2D, arrows). Because TDP-43-mediated sub-nuclear foci formation is important for IL-6 production [9], we specifically examined TDP-43-enriched foci in pre-adipocytes. Notably, TDP-43 foci were clearly observed in pre-adipose cells in the presence or absence of lipopolysaccharide (LPS) stimulation (Fig. 2E), demonstrating that TDP-43 expression is strongly associated with IL-6 production in pre-adipose and adipose cells.

Because adipose tissue also contains immune cells, including macrophages, we also explored if increased TDP-43 expression affected IL-6 production in macrophages. When TDP-43 was overexpressed in RAW macrophages, IL-6 levels were significantly increased in culture supernatants following LPS stimulation compared to supernatants from non-TDP-43-overexpressing cells (Supplementary Fig. 1A). To analyze IL-6 mRNA levels in TDP-43-overexpressing macrophages, we performed qRT-PCR and observed that elevated TDP-43 expression led to increased IL-6 mRNA levels (Supplementary Fig. 1B). Taken together, these data suggest that IL-6 expression levels are strongly dependent on the expression of TDP-43 in various cell types within adipose tissue.

### 3.3. Effect of in vivo TDP-43 depletion on liver inflammation

Previous reports have shown that obesity is linked to hepatic inflammation and insulin resistance [13,14]. In addition, liver tissue releases large amounts of IL-6, suggesting that the liver and adipose tissue are major sources of IL-6 production [15,16]. Because we observed a strong correlation between TDP-43 overexpression and IL-6 levels in obese mice, we investigated the physiological relevance of TDP-43 expression during liver inflammation. We induced *in vivo* TDP-43 depletion and subsequent liver inflammation in the mice by intraperitoneal injection with LPS (Fig. 3A, left) and observed significantly depleted TDP-43 in the liver (Fig. 3A, right). Notably, TDP-43-depleted mice

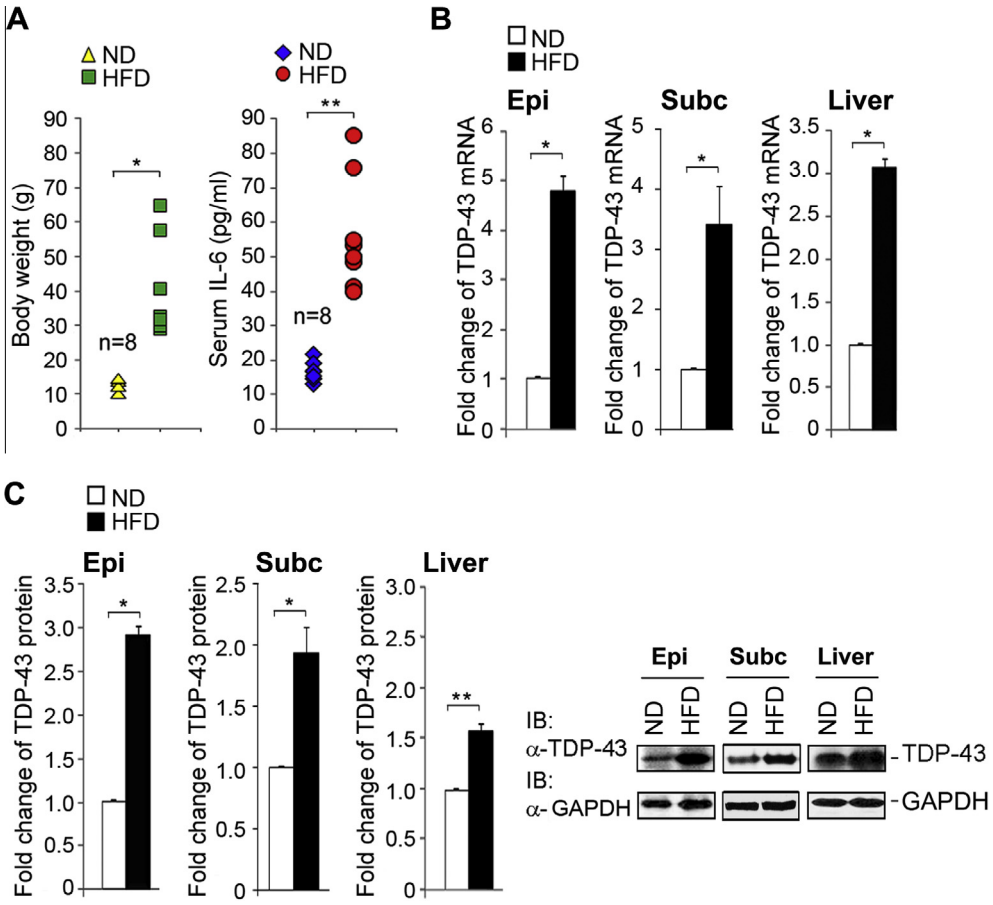


**Fig. 1.** Elevated expression of TDP-43 and IL-6 in obesity. C57BL/6N mice were divided into two groups: control mice fed a normal diet (ND) and experimental mice fed a high-fat diet (HFD). (A) The body weights of these mice were measured at 12 weeks, and serum IL-6 levels from HFD or ND mice were measured by ELISA ($n = 8$). $^{*}P < 0.01$, $^{**}P < 0.05$ (Student's $t$-test). (B and C) Expression of TDP-43 in HFD or ND mice. TDP-43 mRNA or protein expression in the epididymal (Epi), subcutaneous fat (Subc), and liver tissues of HFD or ND mice was assessed by RT-PCR (B) or immunoblot (C) analysis ($n = 8$). $^{*}P < 0.005$, $^{**}P < 0.01$ (Student's $t$-test). Data are representative of at least three independent experiments (mean ± S.D. in A–C).

exhibited reduced IL-6 mRNA production in the liver compared to wild-type mice (Fig. 3B). Furthermore, IL-6 RNA processing was markedly impaired by *in vivo* TDP-43 depletion, even after induction of liver inflammation (Fig. 3C). In addition, levels of C-reactive protein mRNA, primarily induced by IL-6-dependent liver inflammation, were also decreased in the liver of TDP-43-depleted mice (Supplementary Fig. 2A). Our findings related to *in vivo* TDP-43-depletion are consistent with our *in vitro* data from the cell-base analysis. Thus, we conclude that TDP-43 overexpression promotes liver inflammation by accelerating IL-6 mRNA production.

## 4. Discussion

In this study, we showed that aberrant TDP-43 overexpression leads to IL-6 overproduction in obese mice, thereby suggesting that TDP-43 plays a critical role in homeostasis of IL-6 production. Although the loss of nuclear TDP-43 function by its mislocalization in the cytoplasm and by mutations in the *TARDBP* gene is associated with neurodegenerative diseases [17], it is not clear whether altered TDP-43 expression is linked to immunological pathogenesis. Considering the self-regulation of TDP-43 levels via a negative feedback loop [10], this study strongly supports the notion that control of TDP-43 is important to prevent IL-6-dependent pathological conditions.

Cytokines are used in multiple signaling pathways to elicit their biological effects, and cytokine signals are rapidly attenuated to maintain cellular processes and avoid toxic side effects. In particular, IL-6 is an important mediator involved in the regulation of the acute-phase response to injury and infection, neuronal regeneration, embryonic development, and fertility [18–21]. Thus, dysregulation of IL-6 signaling leads to the onset and maintenance of several pathological diseases. Specifically, IL-6 levels are significantly high in obese patients [8]. Obesity-associated increases in IL-6 levels are linked to decreased insulin-induced glucose uptake [22], and IL-6 decreases insulin sensitivity by suppressing adiponectin mRNA synthesis and secretion [23]. Therefore, elevated TDP-43 and IL-6 levels in HFD mice implicate that TDP-43 is associated with the obesity-related conditions. Previous reports have shown that TDP-43 regulates Tbc1d1, a key regulator of glucose homeostasis and obesity, and that TDP-43 overexpression increases Tbc1d1 mRNA levels in skeletal muscle cells [24,25]. However, overexpressed TDP-43 does not affect Tbc1d1 mRNA expression levels in adipose tissues, [25], nor does *in vivo* TDP-43 depletion influence Tbc1d1 mRNA expression in the liver of mice (Supplementary Fig. 2B). These results suggest that differential regulation of TDP-43 in various cell types influences its role during obesity.

Adipose tissue contains adipocytes, pre-adipocytes, endothelial cells, fibroblasts, leukocytes, and macrophages. Adipocytes produce IL-6 during obesity, and pre-adipocytes from stromal vascular fractions significantly contribute to obesity-induced IL-6 expression [26]. Macrophages are also central mediators of obesity-associated inflammation. Therefore, the effect of TDP-43 on IL-6 levels in these cells suggests TDP-43's involvement in obesity-induced pathogenic

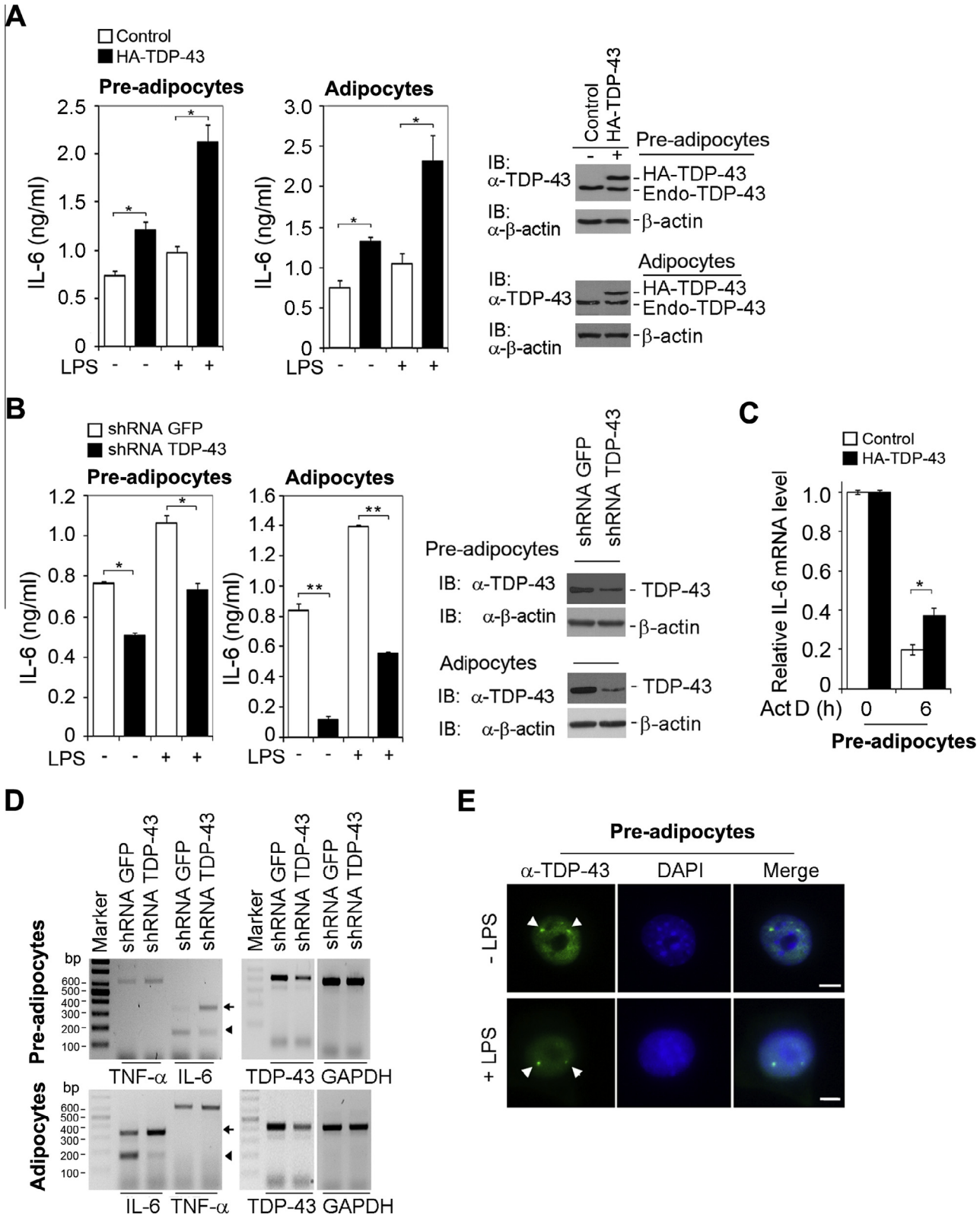


**Fig. 2.** TDP-43-dependent IL-6 expression in pre-adipocytes and adipocytes. (A) Levels of IL-6 in control or HA-TDP-43-overexpressing pre-adipocytes and adipocytes were analyzed by ELISA. $^{*}P < 0.01$ (Student's *t*-test). Levels of TDP-43 were analyzed by immunoblot using anti-TDP-43 antibodies. (B) ELISA was performed to measure IL-6 levels from control or TDP-43-depleted pre-adipocytes and adipocytes. $^{*}P < 0.01$, $^{**}P < 0.005$ (Student's *t*-test). Levels of TDP-43 were confirmed by immunoblot analysis using anti-TDP-43 antibodies. (C) The effect of TDP-43 overexpression on mRNA stability in pre-adipocytes was assessed by qRT-PCR analysis. RNA was isolated at indicated time points after actinomycin D (Act D) treatment. $^{*}P < 0.01$ (Student's *t*-test). (D) Effect of TDP-43 knockdown on cytokine RNA processing in pre-adipocytes and adipocytes. Primer pairs for the exon–exon junction of the indicated cytokines were used. TDP-43 knockdown and quantity of total RNA were confirmed by RT-PCR using the indicated primers (Table 1). Arrowheads and arrows indicate mRNA and pre-mRNA, respectively. (E) Immunofluorescence assays analyzed pre-adipocytes after treatment with or without LPS. Cells were stained with anti-TDP-43 antibodies, and DAPI was used as a nuclear counterstain. Scale bars, 5 μm. Data are representative of at least three independent experiments (mean ± S.D. in A–C).

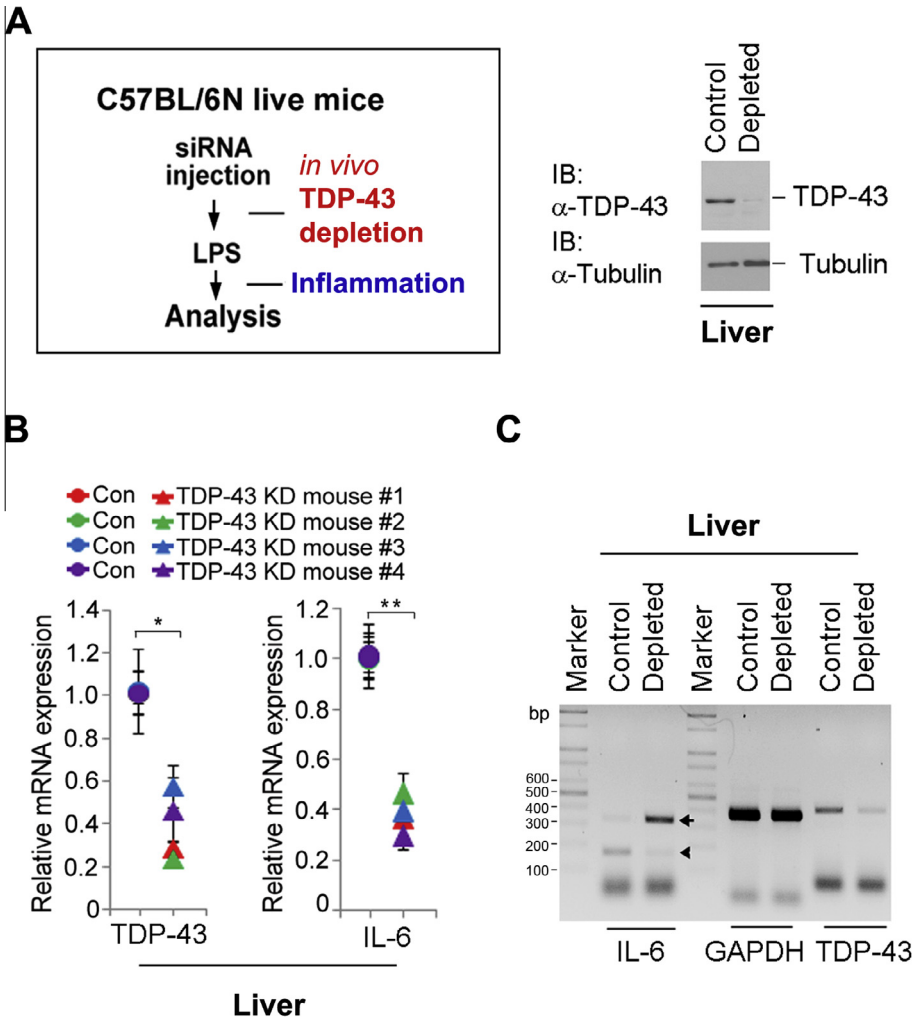


**Fig. 3.** Effect of *in vivo* TDP-43 depletion on liver inflammation. (A) Strategy for depletion of TDP-43 *in vivo* and induction of liver inflammation by LPS. C57BL/6N mice were divided into two different groups: control mice injected with the control-Invivofectamine complex ($n = 4$) and experimental mice injected with the TDP-43 siRNA-Invivofectamine complex ($n = 4$). The efficiency of TDP-43 depletion was confirmed by immunoblot analysis using anti-TDP-43 antibodies. (B) qRT-PCR revealed a significant reduction in IL-6 mRNA production. All qRT-PCR data were normalized to the expression of GAPDH mRNA ($n = 4$). $^{*}P < 0.01$, $^{**}P < 0.005$ (Student's *t*-test). (C) RT-PCR using primer pairs for the exon 1–exon 2 junction of IL-6 RNA was performed on control or TDP-43-depleted mice. Confirmation of TDP-43 knockdown and measurement of the quantity of RNA were confirmed by RT-PCR. Arrowheads and arrows indicate mRNA and pre-mRNA, respectively. Data are representative of at least three independent experiments (mean ± S.E.M. in B).

conditions. In addition, obesity leads to hepatic inflammation and IL-6 over-production [14,27]. Our results show that alteration of TDP-43 expression obviously affects IL-6 expression in the liver, suggesting a potential link between TDP-43 and liver inflammation during obesity.

In conclusion, we observed regulation of IL-6 expression via TDP-43 and TDP-43's involvement in the pathogenesis of obesity. Because IL-6 influences obesity pathogenesis and affects disease severity, defective regulation of IL-6 via aberrant TDP-43 expression could serve as a reliable indicator of certain inflammatory diseases. Based on our findings, these diseases could also be characterized by excessive TDP-43 expression. Although questions remain regarding TDP-43's complete role in obesity, our study provides valuable insight into the molecular pathology of obesity and may contribute to the development of improved therapies for treating obesity-related diseases.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2015.05.040.

## References

[1] Buratti, E. and Baralle, F.E. (2008) Multiple roles of TDP-43 in gene expression, splicing regulation, and human disease. Front. Biosci. 13, 867–878.
[2] Ou, S.H., Wu, F., Harrich, D., Garcia-Martinez, L.F. and Gaynor, R.B. (1995) Cloning and characterization of a novel cellular protein, TDP-43, that binds to human immunodeficiency virus type 1 TAR DNA sequence motifs. J. Virol. 69, 3584–3596.
[3] Wang, H.Y., Wang, I.F., Bose, J. and Shen, C.K. (2004) Structural diversity and functional implications of the eukaryotic TDP gene family. Genomics 83, 130–139.

[4] Buratti, E., Dork, T., Zuccato, E., Pagani, F., Romano, M., et al. (2001) Nuclear factor TDP-43 and SR proteins promote in vitro and in vivo CFTR exon 9 skipping. EMBO J. 20, 1774–1784.
[5] Arai, T., Hasegawa, M., Akiyama, H., Ikeda, K., Nonaka, T., et al. (2006) TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem. Biophys. Res. Commun. 351, 602–611.
[6] Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., et al. (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314, 130–133.
[7] Igaz, L.M., Kwong, L.K., Chen-Plotkin, A., Winton, M.J., Unger, T.L., et al. (2009) Expression of TDP-43 C-terminal fragments in vitro recapitulates pathological features of TDP-43 proteinopathies. J. Biol. Chem. 284, 8516–8524.
[8] Roytblat, L., Rachinsky, M., Fisher, A., Greemberg, L., Shapira, Y., et al. (2000) Raised interleukin-6 levels in obese patients. Obes. Res. 8, 673–675.
[9] Lee, S., Lee, T.A., Lee, E., Kang, S., Park, A., et al. (2015) Identification of a subnuclear body involved in sequence-specific cytokine RNA processing. Nat. Commun. 6, 5791.
[10] Ayala, Y.M., De Conti, L., Avendano-Vazquez, S.E., Dhir, A., Romano, M., et al. (2011) TDP-43 regulates its mRNA levels through a negative feedback loop. EMBO J. 30, 277–288.
[11] Bastard, J.P., Jardel, C., Bruckert, E., Blondy, P., Capeau, J., et al. (2000) Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. J. Clin. Endocrinol. Metab. 85, 3338–3342.
[12] Sopasakis, V.R., Sandqvist, M., Gustafson, B., Hammarstedt, A., Schmelz, M., et al. (2004) High local concentrations and effects on differentiation implicate interleukin-6 as a paracrine regulator. Obes. Res. 12, 454–460.
[13] Arkan, M.C., Hevener, A.L., Greten, F.R., Maeda, S., Li, Z.W., et al. (2005) IKK-beta links inflammation to obesity-induced insulin resistance. Nat. Med. 11, 191–198.
[14] Cai, D., Yuan, M., Frantz, D.F., Melendez, P.A., Hansen, L., et al. (2005) Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat. Med. 11, 183–190.
[15] Norris, C.A., He, M., Kang, L.I., Ding, M.Q., Radder, J.E., et al. (2014) Synthesis of IL-6 by hepatocytes is a normal response to common hepatic stimuli. PLoS One 9, e96053.
[16] Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D.R., Miles, J.M., et al. (1997) Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J. Clin. Endocrinol. Metab. 82, 4196–4200.
[17] Forman, M.S., Trojanowski, J.Q. and Lee, V.M. (2007) TDP-43: a novel neurodegenerative proteinopathy. Curr. Opin. Neurobiol. 17, 548–555.
[18] Kopf, M., Baumann, H., Freer, G., Freudenberg, M., Lamers, M., et al. (1994) Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature 368, 339–342.
[19] Hirano, T., Taga, T., Matsuda, T., Hibi, M., Suematsu, S., et al. (1990) Interleukin 6 and its receptor in the immune response and hematopoiesis. Int. J. Ceell Cloning 8 (Suppl. 1), 155–166. discussion 166–157.
[20] Motro, B., Itin, A., Sachs, L. and Keshet, E. (1990) Pattern of interleukin 6 gene expression in vivo suggests a role for this cytokine in angiogenesis. Proc. Natl. Acad. Sci. USA 87, 3092–3096.
[21] Scheller, J., Chalaris, A., Schmidt-Arras, D. and Rose-John, S. (2011) The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim. Biophys. Acta 1813, 878–888.
[22] Kern, P.A., Ranganathan, S., Li, C., Wood, L. and Ranganathan, G. (2001) Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am. J. Physiol. Endocrinol. Metab. 280, E745–E751.
[23] Fasshauer, M., Kralisch, S., Klier, M., Lossner, U., Bluher, M., et al. (2003) Adiponectin gene expression and secretion is inhibited by interleukin-6 in 3T3-L1 adipocytes. Biochem. Biophys. Res. Commun. 301, 1045–1050.
[24] Chiang, P.M., Ling, J., Jeong, Y.H., Price, D.L., Aja, S.M., et al. (2010) Deletion of TDP-43 down-regulates Tbc1d1, a gene linked to obesity, and alters body fat metabolism. Proc. Natl. Acad. Sci. USA 107, 16320–16324.
[25] Stallings, N.R., Puttaparthi, K., Dowling, K.J., Luther, C.M., Burns, D.K., et al. (2013) TDP-43, an ALS linked protein, regulates fat deposition and glucose homeostasis. PLoS One 8, e71793.
[26] Harkins, J.M., Moustaid-Moussa, N., Chung, Y.J., Penner, K.M., Pestka, J.J., et al. (2004) Expression of interleukin-6 is greater in preadipocytes than in adipocytes of 3T3-L1 cells and C57BL/6J and ob/ob mice. J. Nutr. 134, 2673–2677.
[27] Park, E.J., Lee, J.H., Yu, G.Y., He, G., Ali, S.R., et al. (2010) Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. Cell 140, 197–208.