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Inhibitory effect of p53 on mitochondrial content and function during adipogenesis

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ABSTRACT

The p53 protein is known as a guardian of the genome and is involved in energy metabolism. Since the metabolic system is uniquely regulated in each tissue, we have anticipated that p53 also would play differential roles in each tissue. In this study, we focused on the functions of p53 in white adipose tissue (adipocytes) and skeletal muscle (myotubes), which are important peripheral tissues involved in energy metabolism. We found that in 3T3-L1 preadipocytes, but not in C2C12 myoblasts, p53 stabilization or overexpression downregulates the expression of *Ppargc1a*, a master regulator of mitochondrial biogenesis. Next, by using p53-knockdown C2C12 myotubes or 3T3-L1 preadipocytes, we further examined the relationship between p53 and mitochondrial regulation. In C2C12 myoblasts, p53 knockdown did not significantly affect *Ppargc1a* expression and mtDNA, but did suppress differentiation to myotubes, as previously reported. However, in 3T3-L1 preadipocytes and mouse embryonic fibroblasts, p53 downregulation enhanced both differentiation into adipocytes and mitochondrial DNA content. Furthermore, p53-depleted 3T3-L1 cells showed increase in mitochondrial proteins and enhancement of both Citrate Synthase and Complex IV activities during adipogenesis. These results show that p53 differentially regulates cell differentiation and mitochondrial biogenesis between adipocytes and myotubes, and provide evidence that p53 is an inhibitory factor of mitochondrial regulation in adipocyte lineage.

1. Introduction

Tumor suppressor p53, which is known as a genome guardian that functions in various intrinsic and extrinsic stresses, is one such protein [1]. Under stress conditions, p53 is stabilized and activated via post-translational modifications such as phosphorylation and acetylation, whereas under non-stress conditions, p53 protein is maintained at low levels by proteasomal degradation via ubiquitination involving ubiquitin E3 ligases such as MDM2 [1]. In the last decade, diverse physiological functions of p53 have been reported. Regulation of metabolic pathways such as glycolysis, oxidative phosphorylation, and fatty acid oxidation are among these functions of p53 [2]. Furthermore it has been shown that p53 regulates mitochondrial function, which is closely involved in metabolic regulation [3,4]. An important regulatory system of mitochondrial function is mitochondrial biogenesis. In most cases, mitochondrial biogenesis is organized by a transcriptional coactivator PGC1α, which is a protein product of *Ppargc1a* and upregulates nuclear genes encoding mitochondrial proteins via enhancement of mRNA expression of transcriptional factors such as NRF1 and NRF2 [5]. It has been reported previously that p53 positively or negatively regulates *Ppargc1a* expression according to each tissue or cell type [6–9]. Furthermore, it has been reported that p53 deficient fibroblasts show low mtDNA copy number [10], and p53 accumulation in neonatal cardiomyocytes leads to mitochondrial impairment [8]. Thus, although there are emerging evidences for links between p53 and mitochondrial biogenesis, the distinct physiological roles in each tissue type remain unclear.

Control of cell differentiation is also regarded as one of the diverse physiological functions of p53. For example, it was recently

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reported that in the process of reprogramming differentiated cells into induced pluripotent stem cells, p53 functions as a barrier to dedifferentiation [11]. Also, in the field of cell differentiation, roles for p53 in adipogenesis and myogenesis have been reported. mouse embryonic fibroblasts (MEFs) or bone marrow-derived mesenchymal stem cells (MSCs) derived from p53 knockout (KO) mice and p53 knockdown 3T3-L1 preadipocytes differentiate into adipocytes more efficiently than p53 proficient cells [12–14]. In C2C12 myoblasts p53 knockdown or p53 mutant expression inhibits its differentiation to myotubes [12,15,16]. Thus, the roles of p53 in cell differentiation differ according to cell types.

Investigation of individual tissues involved in energy metabolism appeared to be important to elucidate the diversity of the metabolic roles of p53 in energy metabolism within the whole body. Therefore in this study, we selected adipose tissue and skeletal muscle, which are peripheral metabolic tissues, as the target tissues. Adipose tissue, which is mainly composed of adipocytes, functions as an organ for energy storage and release *via* lipid metabolism. Skeletal muscle, which is mainly composed of myotubes, plays a central role for basal metabolism in the whole body. Using 3T3-L1 preadipocytes and MEFs as the origin cells of adipocytes and C2C12 myoblasts as the origin cells of myotubes, we found that p53 has a differential contribution to mitochondrial biogenesis and cell differentiation in each cell type. Our results imply that p53 is a key factor that reflects on tissue-specific diversity in metabolism.

2. Materials and methods

2.1. Cell lines and drugs

3T3-L1 preadipocytes were purchased from RIKEN Bioresource Center (Ibaraki, Japan) and 3T3-L1/shGFP and 3T3-L1/shp53 preadipocytes were previously established by our laboratory using a retrovirus system [17,18]. Primary mouse embryonic fibroblasts (MEFs) derived from p53 WT and p53 KO mice (RIKEN Bioresource Center) were established as previously reported by our laboratory [19]. C2C12 myoblasts were kindly provided by Dr. Kazuhiro Shigemoto. C2C12/shGFP and C2C12/shp53 myoblasts were established as the same retroviral systems used for 3T3-L1/shGFP and 3T3-L1/shp53. Nutlin-3a was supplied by Cayman (MI, USA).

2.2. Cell culture and differentiation

3T3-L1 preadipocytes were maintained in DMEM (low glucose) (Wako Pure Chemical; Osaka, Japan) with 10% fetal calf serum (FCS) (Bovogen Biologicals; Victoria, Australia) and 1% penicillin/streptomycin (Sigma; MO, USA). MEFs were grown in DMEM (high glucose) (Wako Pure Chemical) with 10% FCS, 0.1 mM 2-mercaptoethanol, and 1% penicillin/streptomycin. Differentiation of 3T3-L1 preadipocytes or MEFs to adipocytes was performed as previously reported by our laboratory [18]. C2C12 myoblasts were maintained in DMEM high glucose supplemented with 10% FCS and antibiotics. Differentiation of C2C12 myoblasts to myotubes was performed using horse serum essentially as previously reported [20].

2.3. Western blotting

Western blotting was performed as previously reported by our laboratory [18,19,21] with the following primary antibodies: anti-p53 monoclonal antibody (clone Ab-1; Calbiochem; CA, USA), anti-β actin monoclonal antibody (clone AC-15; Sigma), or anti-α tubulin monoclonal antibody (clone DM1A; Sigma), anti-PPARγ polyclonal antibody (E-8; Santa Cruz Biotechnology; CA, USA), anti-FABP4 polyclonal antibody (Cayman), anti-COXIV polyclonal antibody (Cell Signaling Technology; MA USA), anti-TOM20 (clone 4S3; Sigma).

2.4. Oil Red O staining

Oil Red O staining was performed as previously reported by our laboratory [18,19]. The stained cells were observed by a BIOREVO BZ-9000 microscope (Keyence; Osaka, Japan).

2.5. RNA purification and RT-PCR

RNA purification and RT-PCR were performed essentially as previously reported by our laboratory [19,21]. Total RNA was extracted from cells using RNAiso PLUS (TaKaRa; Shiga, Japan) and purified with a FastPure RNA kit (TaKaRa) according to the manufacturer's protocol. The purified RNA was subjected to reverse transcription with PrimeScript Reverse Transcriptase (TaKaRa) and random hexamer (TaKaRa). The semi-quantitative RT-PCR was performed using Platinum Taq DNA Polymerase High Fidelity (Invitrogen). Quantitative RT-PCR was performed using the Applied Biosystems 7300 real-time PCR system (Applied Biosystems; CA, USA) and SYBR Premix Ex Taq II (TaKaRa) according to the manufacturer's protocol. The sequences of primers used for RT-PCR are shown in Table 1.

2.6. Mitochondrial DNA (mtDNA) content

mtDNA content was evaluated essentially as previously reported by our laboratory [21]. Briefly, extracted total DNA was subjected to real-time PCR using COXII primers (forward, 5′-CCATCCCAGGCCGACTAA-3′; reverse, 5′-AATTTCAGAGCATTGGCCATAGA-3′) or β-Globin primers (forward, 5′-ATCCAGGTTACAAGGCAGCT-3′; reverse, 5′-GGGAAACATAGACAGGGG-3′). The relative mitochondrial copy number is represented by the ratio of COXII encoded in mtDNA to β-Globin encoded in genomic DNA.

2.7. Citrate synthase (CS) and Complex IV activity

To prepare cell lysates for the measurement of mitochondrial activity, 3T3-L1/shGFP and 3T3-L1/shp53 were homogenized in homogenization buffer containing 50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1% phosphatase inhibitor cocktail, 5 mM EDTA, 1% protease inhibitor cocktail, 1% NP-40 and 0.05% sodium deoxycholate. Protein concentration was determined using the BCA protein assay kit (Thermo Scientific; IL, USA) according to the manufacturer's protocol.

CS activity was measured by monitoring color development of thio-bis-(2-nitrobenzoic) acid (TNB) generated from reduction of 5,5-dithio-bis-(2-nitrobenzoic) acid (DTNB) by CoA-SH, the by-product of citrate, as previously reported [21]. Briefly, cell lysates were added to reaction mixtures containing 0.1 mM DTNB, 0.5 mM acetyl-CoA, 0.1% Triton X-100 and 100 mM Tris–HCl, pH 8.0. After incubation at 25 °C for 5 min, reactions were then initiated by the addition of 0.5 mM oxaloacetate in a final volume of 200 μL, and the change in absorbance at 412 nm was recorded for at least 3 min using ARVO MX/Light Wallac 1420 Multilabel/Luminescence Counter (PerkinElmer; MA, USA).

Complex IV activity was measured by monitoring the change in absorbance at 550 nm of cytochrome *c* by oxidation as previously reported [21]. Briefly, reactions were initiated by adding cell homogenates to the reaction mixture containing 50 mM Tris–HCl, pH 7.2, and 25 μM reduced cytochrome *c*. The absorbance at 550 nm (at 30 °C) was measured using EnVision Multilabel Reader (PerkinElmer). The first order rate constant (k) was calculated from

Table 1
Sequences of primers used in RT-PCR.

Gene	Forward sequence	Reverse sequence
Actb	5′-TCTTTGCAGCTCCTTCGTTG-3′	5′-GGCCTCGTCACCCACATAG-3′
Fabp4	5′-TCGATGATTACATGAAAGAAGTGG-3′	5′-CGCCCAGTTTGAAGGAAATC-3′
Gusb	5′-CCAGAGCGAGTATGGAGCAGAC-3′	5′-GGTGACTGGTTCGTCATGAAGTC-3′
Myod	5′-CTGCTCTGATGGCATGATGG-3′	5′-TATGCTGGACAGGCAGTCG-3′
Myog	5′-ATCCAGTACATTGAGCGCCTAC-3′	5′-TGTCCACGATGGACGTAAGG-3′
p21	5′- AGTACTTCCTCTGCCCTGCTG -3′	5′-GCGCTTGGAGTGATAGAAATCTG-3′
p53	5′-TAAAGGATGCCCATGCTACAG-3′	5′-GACCGGGAGGATTGTGTCTC-3′
Pgc1a	5′-AGACGGATTGCCCTCATTTG-3′	5′-CAGGGTTTGTTCTGATCCTGTG-3′
Pparg	5′-CACAATGCCATCAGGTTTGG-3′	5′-GCGGGAAGGACTTTATGTATGAG-3′
Rps18	5′-TGCGAGTACTCAACACCAACAT-3′	5′-CTTTCCTCAACACCACATGAGC-3′
Tbp	5′-CAGTACAGCAATCAACATCTCAGC-3′	5′-CAAGTTTACAGCCAAGATTCACG-3′

the natural logarithms of the absorbance after the addition of cell homogenates.

2.8. Statistical analysis

All statistical analyses (Student's *t*-test with or without Bonferroni correction) were performed using R software (R project for Statistical Computing). Differences with p values <0.05 were deemed statistically significant.

3. Results

3.1. Distinct effects of p53 on Ppargc1a expression in 3T3-L1 preadipocytes and C2C12 myoblasts

By using Nutlin-3a, which is a chemical inhibitor of MDM2 E3 ligase [22,23], when examining metabolic regulation by DNA damage-independent p53 stabilization in adipocytes and myotubes, we obtained interesting results. In both C2C12 myoblasts (Fig. 1A, left panel) and 3T3-L1 preadipocytes (Fig. 1B, left panel) Nutlin-3a induced p53 protein accumulation and p21 mRNA expression in a dose dependent manner. Interestingly, in 3T3-L1 preadipocytes (Fig. 1B, right panel) but not in C2C12 myotubes (Fig. 1A, right panel), *Ppargc1a* expression was decreased in a dose dependent manner. To exclude side-effects of Nutlin-3a, we examined whether p53 exogenous overexpression decreases *Ppargc1a* expression. Consistent with Nutlin-3a treatment, p53 overexpression in 3T3-L1 preadipocytes (Fig. 1D) but not C2C12 myoblasts (Fig. 1C) reduced *Ppargc1a* expression significantly. Next, to investigate the effects of p53 downregulation on *Ppargc1a* expression, we used p53-knockdown (KD) C2C12 myoblasts and 3T3-L1 preadipocytes (Fig. 1E and F, left panel). As shown in Fig. 1E and F, no significant change of *Ppargc1a* expression occurred in either of the cell lines. In these experimental conditions, although we analyzed the protein levels of PGC1α, a protein product of *Ppargc1a*, by Western blotting using two commercially available anti-PGC1α antibodies (Santa Cruz Biotechnology, Cat. No. sc-13067 and Merk-Millipore, Cat. No. ST1202-1SET), we were not, unfortunately, able to detect any specific signals (data not shown). These results indicate that, p53 up-regulation in 3T3-L1 preadipocytes but not C2C12 myoblasts reduces *Ppargc1a* expression.

3.2. Effects of p53 downregulation on Ppargc1a expression and mtDNA content in myotubes differentiated from C2C12 myoblasts

Previous studies have suggested that p53 plays a positive regulatory role in mitochondrial regulation in skeletal muscle and myotubes [6,24]. However, our data indicated that, at least in C2C12 myoblasts, which were established from skeletal muscle [25], p53 may play an opposing role (Fig. 1). We therefore sought to investigate the involvement of p53 in mitochondrial regulation in myotubes differentiated from myoblasts (Fig. 2). Several studies have shown that p53 positively regulates differentiation of C2C12 myoblasts into myotubes [12,15,16]. Consistent with the previous studies, p53 knockdown inhibited multinucleated myotube formation slightly (Fig. 2A) and tended to reduce the mRNA expression of differentiation markers (*Myog* and *Myod*) during myogenesis compared with control cells (data not shown). Interestingly, we found that *Ppargc1a* expression (Fig. 2B) and mtDNA content (Fig. 2C) were not affected by p53 downregulation, even in myotubes. These results indicate that p53 does not affect mitochondrial regulation in myotubes, although differentiation of C2C12 myoblasts to myotubes is suppressed by p53 knockdown.

3.3. Effects of p53 downregulation on Ppargc1a expression and mtDNA content in adipocytes derived from 3T3-L1 preadipocytes and MEFs

As shown in Fig. 1, it seemed to suggest that p53 is a negative regulator of mitochondrial biogenesis in 3T3-L1 preadipocytes. Because previous studies have reported that mitochondrial biogenesis is upregulated during adipogenesis of 3T3-L1 preadipocytes [26,27], we sought to confirm the effects of p53 knockdown on signs of mitochondrial biogenesis during adipogenesis. The results of Oil Red O staining showed that p53 KD leads to efficient accumulation of lipid droplets (Fig. 3A). Consistent with this result, the mRNA expressions of adipogenesis markers (*Pparg and Fabp4*) during differentiation of 3T3-L1/shp53 preadipocytes to adipocytes tended to increase compared with control (data not shown). Similar to the result in preadipocytes (Fig. 1), we found that p53-knockdown mature adipocytes have significantly increased *Ppargc1a* expression (Fig. 3B). Interestingly, since Day 8, mtDNA content in p53-knockdown cells significantly increased compared with those in mock cells (Fig. 3C). Furthermore we quantitatively analyzed protein levels of PPARγ2 (a form of PPARγ protein responsible for adipogenesis) [28] and FABP4, which are protein products of *Pparg* and *Fabp4*, respectively. As shown in Fig. 3D, the protein levels of both PPARγ2 and FABP4 in 3T3-L1/shp53 cells at Day 8 of adipocyte differentiation are significantly higher than in the control. These results are consistent with a previous finding that p53 downregulation promotes adipogenesis in 3T3-L1 preadipocytes [14]. Since p53 downregulation in 3T3-L1 adipocyte-committed cells leads to the enhancement of adipogenesis and increase in mtDNA content, we analyzed whether the similar results were obtained in p53-deficient MEFs, adipocyte-uncommitted cells. Primary MEFs from p53 WT mice and p53 KO mice were differentiated into adipocytes. Oil Red O staining of the differentiated MEFs revealed that adipocytes from p53 KO MEFs accumulated more lipid droplets compared with those from p53 WT MEFs (Fig. 3E). Furthermore, as shown in Supplemental Fig. 1, the mRNA levels of adipocyte differentiation

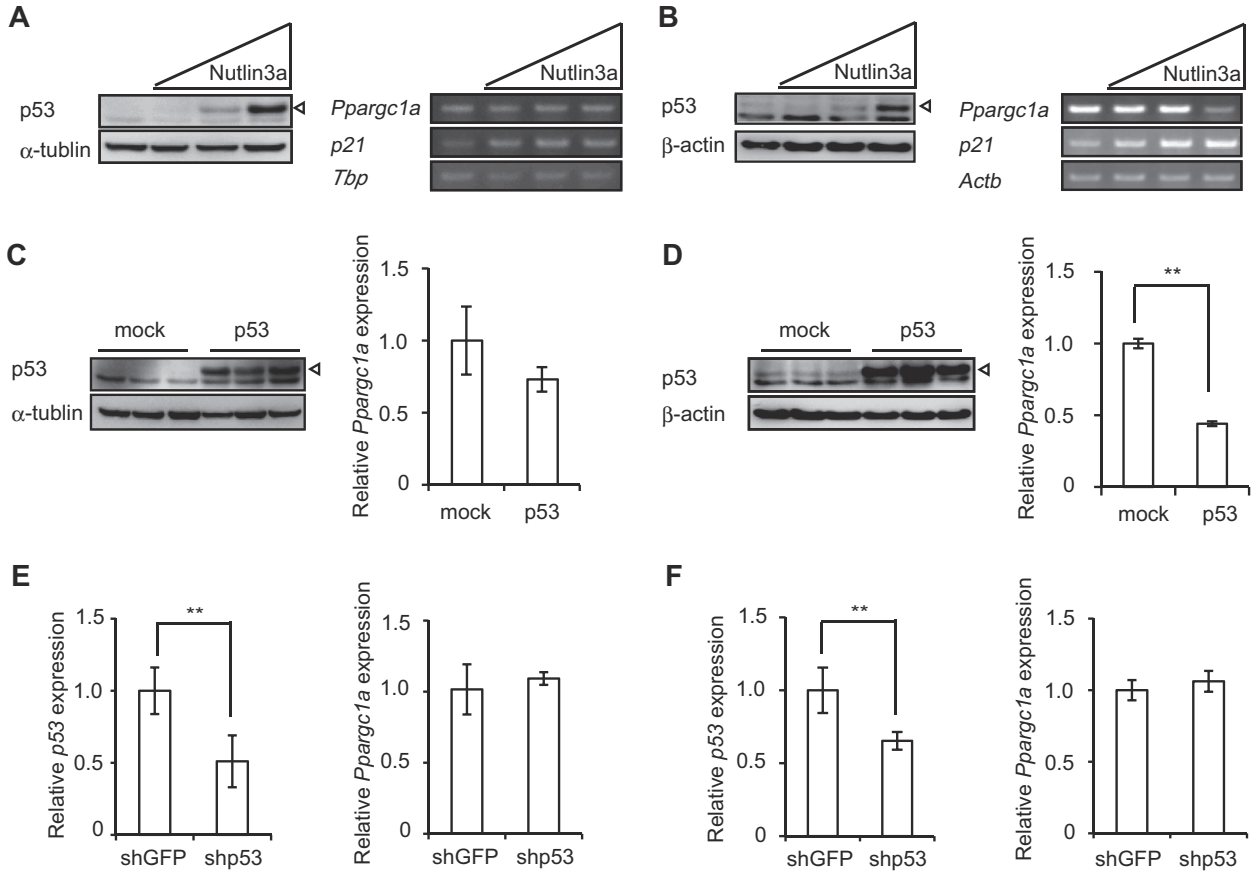

Fig. 1. Differential impacts of p53 on *Ppargc1a* expression in myoblasts and preadipocytes. (A, B) C2C12 myoblasts (A) and 3T3-L1 preadipocytes (B) were treated with 0, 1, 5, or 25 μM of Nutlin3a for 24 h. Protein levels of p53 were analyzed by Western blotting (left panels). Open triangles indicate the signals from p53. α-Tubulin (for C2C12 cells) or β-actin (3T3-L1 cells) was used as a loading control. *Ppargc1*a and *p21* expression levels were analyzed by RT-PCR (right panels). *Tbp* (for C2C12 cells) or *Actb* (for 3T3-L1 cells) was used as a loading control. (C, D) C2C12 myoblasts (C) and 3T3-L1 preadipocytes (D) were transfected with mouse p53 expression (exo-p53) or empty vector (mock). The cells were harvested after 20 h of transfection. Protein levels of p53 were analyzed by Western blotting (left panels). α-Tubulin (C; for C2C12 cells) or β-actin (D; for 3T3-L1 cells) was used as a loading control. (E, F) C2C12 myoblasts (E) and 3T3-L1 preadipocytes (F) were stably transfected with either p53 (shp53) or GFP (shGFP) shRNA expression vectors. *p53* and *Ppargc1a* expression levels were analyzed by quantitative real-time PCR. *Tbp* (E; for C2C12 cells) or *Rps18* (F; for 3T3-L1 cells) was used as a loading control. The quantitative data are represented as means ± SD ($n = 3$) ($^{**}p < 0.01$).

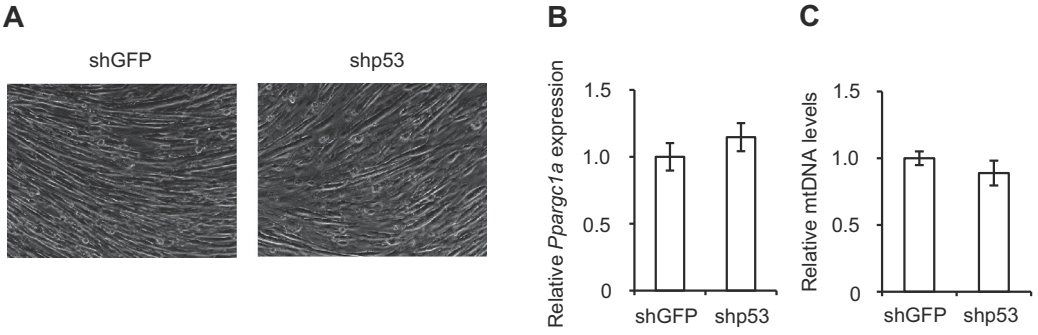

Fig. 2. Effects of p53 downregulation on *Ppargc1a* expression and mtDNA content in myotubes differentiated from C2C12 myoblasts. p53-knockdown (shp53) and control (shGFP) C2C12 myoblasts were differentiated into myotubes (Day 5) and the pictures were taken using a phase contrast microscope (magnification; ×200) (A). *Ppargc1a* expression (B) and mtDNA content (C) in myoblasts (Day 5) were analyzed by quantitative real-time PCR. *Tbp* was used as a reference control. All data reported (means ± SD) were not statistically significant ($n = 3$).

markers appeared to be moderately higher in p53KO MEFs than in p53 WT MEFs. These results were consistent with previously reported findings [12,13]. Next, we examined the effects of p53 on *Ppargc1a* expression and mtDNA content in adipocytes from MEFs using this adipogenesis system. Both the contents of mtDNA and *Ppargc1a* expression in adipocytes at Day16 after differentiation induction increased in p53 KO adipocytes compared with p53 WT adipocytes (Fig. 3F and G). Next, we investigated quantity of proteins that constitute mitochondrion. As shown in Fig. 3H, COX4, a subunit of mitochondrial cytochrome oxidase complex, and TOM20, a subunit of mitochondrial import receptor, increased in p53-knockdown adipocytes compared with control adipocytes.

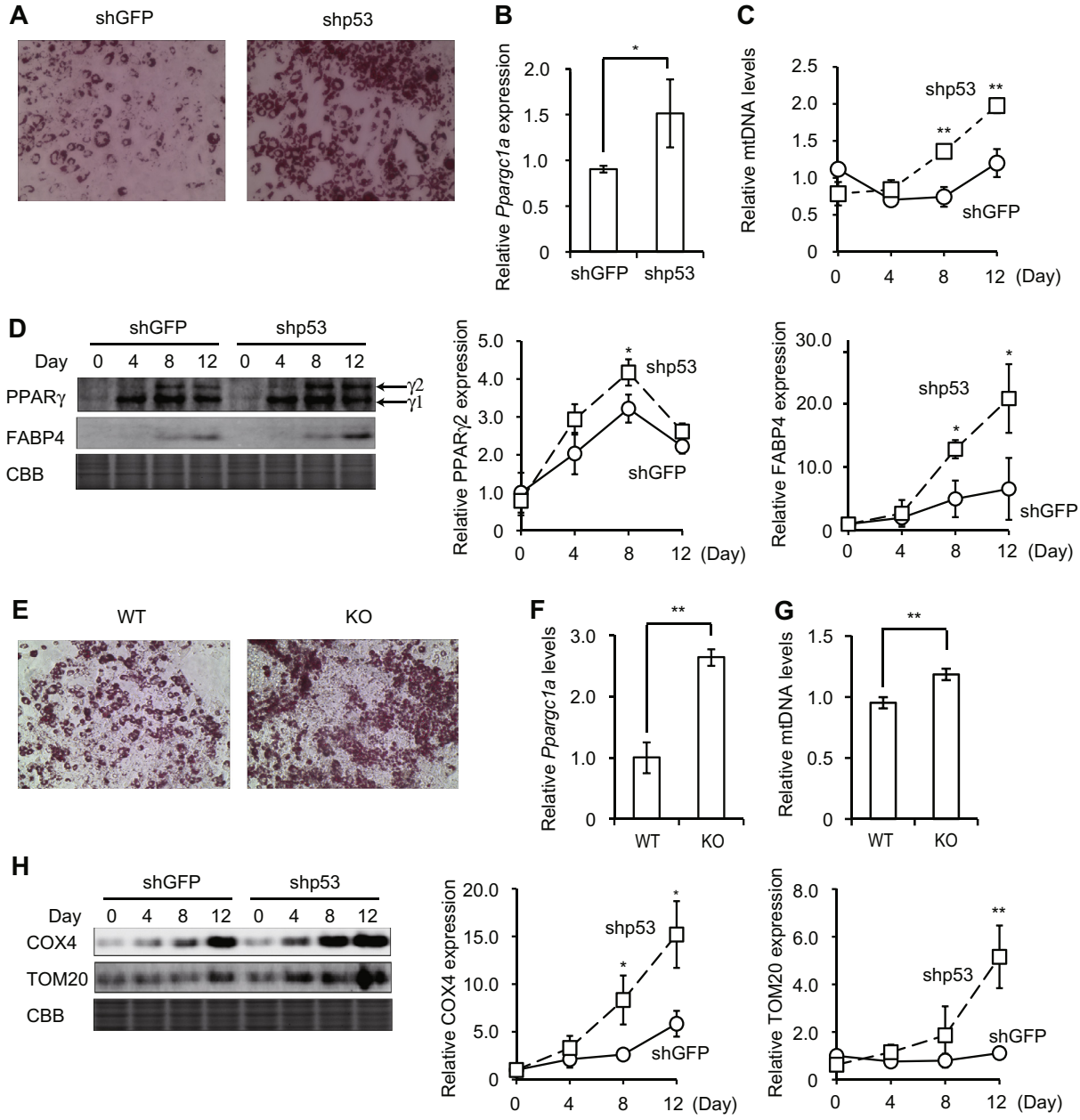

Fig. 3. Effects of p53 downregulation on *Ppargc1a* expression and mtDNA content in adipocytes derived from 3T3-L1 preadipocytes and MEFs. (A) shRNA-mediated p53-knockdown (shp53) and control (shGFP) 3T3-L1 preadipocytes were differentiated into adipocytes (Day 12) and stained with Oil Red O to observe intracellular triglyceride accumulation. (B) *Ppargc1a* expression in adipocytes (Day 12) was analyzed by quantitative real-time PCR. *Rps18* was used as a reference control. (C) Mitochondrial DNAs purified from differentiated adipocytes were analyzed by quantitative real-time PCR. (D) Extracted proteins from each day were subjected to Western blotting with the indicated antibodies. Representative images and the quantitative data ($n = 3$) were shown. CBB staining was used as a loading control. (E) p53 WT and p53 KO MEFs were differentiated to adipocytes (Day 16), and stained with Oil Red O to observe intracellular triglyceride accumulation ($n = 4$). (F, G) *Ppargc1a* expression (F) and mtDNA content (G) in adipocytes (Day 16) were analyzed by quantitative real-time PCR ($n = 3$). *Gusb* was used as a reference control. (H) Extracted proteins from each day were subjected to Western blotting with the indicated antibodies. Representative images and the quantitative data ($n = 3$) were shown. The quantitative data are represented as means ± SD ($n = 3$–5) ($^{*}p < 0.05$; $^{**}p < 0.01$).

These results suggest that p53 affects on mitochondrial content during adipogenesis.

3.4. Effects of p53 downregulation on mitochondrial metabolic function during adipogenesis

Since Fig. 3 showed that p53 downregulation increases mitochondrial content during adipogenesis, we investigated whether the mitochondrial activity increases consistent with the observation. For this purpose, we selected two enzyme activities involved in metabolic pathway existed in mitochondria. As shown in Fig. 4, both CS, a member of TCA cycle, and Complex IV, a member of electron transport chain, activity in p53-knockdown adipocytes significantly increased compared with those in mock cells.

4. Discussion

In the present study, on the ground of increases in mtDNA content and mitochondrial protein accompanying mitochondrial metabolic activity, we demonstrated that p53 is a negative regulator of mitochondrial biogenesis in adipocytes, which is one of peripheral tissues important in energy metabolism. On the other hand, we

have also considered mitochondrial elimination systems during adipogenesis. Autophagy is one of the most important systems for mitochondrial turn-over [29]. Recently our group has reported that autophagosomes accumulate in a p53-independent manner during adipogenesis [18] and anticipate that this phenomenon would result in impairment of autophagy during adipogenesis. Hence, our observation in this study, that p53 possesses an inhibitory effect on mitochondrial content in adipocyte lineage, is unlikely to be related to autophagy-mediated mitochondrial elimination.

By using C2C12 myoblasts, which are derived from skeletal leg muscle of a C3H mouse [30], we also confirmed the contribution of p53 to mitochondrial biogenesis. However, our results showed that p53 did not dramatically affect *Ppargc1a* expression and mtDNA content in C2C12 myoblasts and myotubes (Figs. 1 and 2). In the past, the relationship between p53 and mitochondrial regulation in skeletal muscle has been examined and characterized. *Ppargc1a* expression and mtDNA content of the skeletal muscle of p53 null mice are reduced in comparison with those of WT mice [6,24]. Furthermore, the effects of p53 on mitochondrial biogenesis were greater for slow twitch fiber-rich muscles versus fast twitch fiber-rich muscles [24]. Taken together with the past findings, this finding may imply that C2C12 cells possess features of fast twitch fiber-rich muscle.

What is the molecular mechanism of p53-mediated mitochondrial regulation in adipocyte lineage? This important question may be able to explain by PGC1α-orchestrated mitochondrial biogenesis. The past findings have indicated about two sides of p53-mediated PGC1α regulation [6–9]. Our present study demonstrated that, at least in adipocyte lineage (preadipocyte and mature adipocyte), p53 functions as a negative regulator of *Ppargc1a*. Although it is important to confirm the changes of the protein levels of PGC1α, we were not able to detect endogenous PGC1α protein under any experimental conditions performed in this study. Generally the protein level of PGC1α is very low in white adipose tissue and, even in brown adipose tissue, in which the protein level of PGC1α is relatively high, stimuli such as cold shock is required for the specific detection of PGC1α protein by Western blotting [31,32]. Nevertheless, the study of adipose tissue-specific *Ppargc1a*-disrupted mice revealed evidence that, in the absence of such stimuli, *Ppargc1a* is involved in fatty acid oxidation and tricarboxylic acid cycle in white adipose tissue [32]. This fact implies that the basal expression of *Ppargc1a* can contribute to physiological functions in adipocytes.

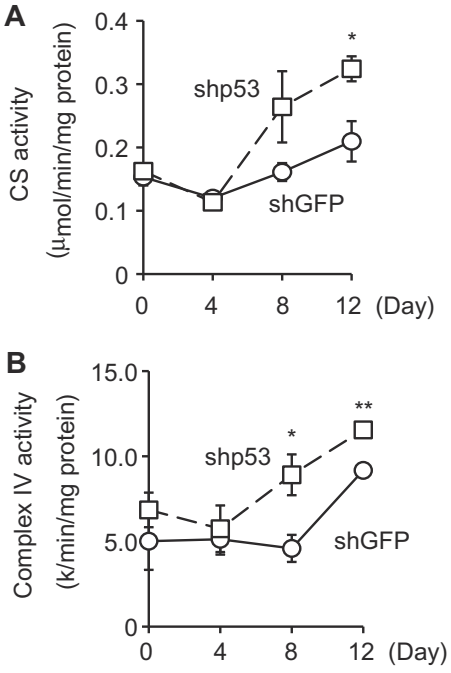

Fig. 4. Effects of p53 downregulation on mitochondrial metabolic function during adipogenesis. CS (A) and Complex IV (B) activities were analyzed as described in Section 2. The quantitative data are represented as means ± SD ($n = 3$) ($^{*}p < 0.05$; $^{**}p < 0.01$).

In this study, we showed that in muscle cells p53 promotes differentiation of C2C12 myoblasts into myotubes, but does not influence mitochondrial biogenesis, whereas in adipocytes p53 inhibits adipogenesis and plays a negative regulatory role in mitochondrial regulation. Recent *in vivo* studies demonstrated the negative effects of p53 on whole body metabolism via adipose tissue, and that adipose tissue-specific p53 depletion suppressed aging and inflammation, and improved insulin sensitivity [33]. Furthermore, mitochondrial content or quality is closely involved in insulin sensitivity [34]. Thus, our *in vitro* study in regard to adipocytes provides evidence of these *in vivo* results.

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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.bbrc.2014.02.059.

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