

p53 Activation in Adipocytes of Obese Mice*

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The tumor suppressor p53 is a transcription factor that activates or represses its target genes after various genotoxic stresses. We have previously shown that sterol regulatory element-binding protein-1 (SREBP-1), a key transcriptional regulator of triglyceride synthesis, and the lipogenic enzymes under its control are markedly suppressed in adipocytes from genetically obese *ob/ob* mice. Here we demonstrate that p53 and its target genes are highly induced in adipocytes of *ob/ob* mice in a fed state, leading to the negative regulation of SREBP-1 and thereby lipogenic genes. In fact, disruption of p53 in *ob/ob* mice completely suppressed the p53-regulated genes to wild-type levels and partially restored expression of lipogenic enzymes. Consistently, reporter gene analysis showed that p53 overexpression suppressed the promoter activity of the *SREBP-1c* gene and its downstream genes. Thus, the activation of p53 might constitute a negative feedback loop against excess fat accumulation in adipocytes. In conclusion, we discovered a novel role of p53 in the pathophysiology of obesity.

Obesity is a major health problem in industrialized societies, affecting ~20–40% of adults (1). The genetically obese *ob/ob* mice develop obesity, insulin resistance, and glucose intolerance owing to an inherited deficiency of the appetite-suppressing hormone, leptin (2–6). The absence of leptin presents the most severe obesity known in both rodents and humans (7), and provides a good model of obesity and its related syndromes, including insulin resistance. Although the underlying mechanisms that link obesity and defective insulin signaling are as yet undefined, hypertrophied adipocyte-derived cytokines such as tumor necrosis factor (TNF)¹- α have been reported to be mediators of insulin resistance in obesity (8, 9).

In the insulin signaling pathways, a transcription factor sterol regulatory element-binding protein-1 (SREBP-1) has recently been established to be a key molecule for the transcriptional regulation of triglyceride synthesis (10). SREBPs are

members of the basic helix-loop-helix leucine zipper family of transcription factors that regulate fatty acid and cholesterol synthesis (reviewed in Refs. 11–13). Whereas SREBP-2 plays a crucial role in regulation of cholesterol synthesis, SREBP-1 controls the transcription and expression of lipogenic enzymes such as fatty acid synthase (FAS) (reviewed in Refs. 14–17). In fact, SREBP-1 and its downstream lipogenic enzymes are drastically induced when fasted animals are refed (18). These lipogenic genes belong to the group of genes that are induced most strongly by glucose/insulin² and can be regarded as indicators of insulin signaling.

We have recently reported that the refeeding responses of SREBP-1 and its downstream lipogenic enzymes are markedly suppressed in adipocytes of *ob/ob* mice, which is presumably associated with impaired insulin signaling (19). Although the precise role of this down-regulation is currently undefined, it could be a negative feedback mechanism to prevent excess fat accumulation in extremely obese animals.

The p53 gene was the first tumor suppressor gene to be identified and has been found to be inactivated in most human cancers (20). The p53 protein is responsible for preventing division of stressed cells and even causes programmed cell death (apoptosis) through activation and/or suppression of the transcription of target genes. For example, γ -irradiation activates p53 to turn on the transcription of p21^{Waf1/CIP1}, which binds to and inhibits cyclin-dependent kinases, thus blocking the G₁-S and G₂-to-mitosis transitions. p53 not only activates transcription of genes such as p21 through its response element, but also represses genes lacking the element by binding to and sequestering essential transcription factors such as TATA-binding protein (21, 22). The stresses that activate p53 are diverse, ranging from DNA damage to oxidative stress, hypoxia, and heat shock (23). The cytostatic and cytotoxic effects of TNF α were also demonstrated to be mediated, at least in part, by p53 activation (24–26). Thus, p53 has been thought to be a guardian angel against cellular stresses. Especially, it has been extensively studied and well established as a tumor suppressor. However, other roles of p53 beyond tumor suppression are still obscure.

Considering that TNF α is relevant to both cell growth and metabolic events and that its effects are partly mediated by p53, we speculated that p53 could be involved in situations of metabolic deterioration associated with insulin resistance. It is possible that p53 as a general repressor of gene transcription could prevent insulin-responsive genes from being activated. Moreover, a previous report on the gene expression profile of *ob/ob* mouse adipose tissue examined by DNA microarray analysis has revealed that p21 and Bax α , both of which are well-

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¹ The abbreviations used are: TNF, tumor necrosis factor; SREBP, sterol regulatory element-binding protein-1; IGFBP-3, insulin-like growth factor-binding protein-3; GPDH, glycerol-3-phosphate dehydrogenase.

² N. Yahagi, unpublished data.

known p53 target genes, are increased from 2- to 3-fold in *ob/ob* mice (27).

Based on these facts, we hypothesized that hypertrophied adipocytes are under various stresses that induce p53, which in turn suppresses lipogenesis in a negative feedback regulation. In our present study, we discovered that p53 is induced upon refeeding in *ob/ob* adipocytes and activates its target genes including p21. In addition, p53 is involved in the suppression of SREBP-1 and the concomitant down-regulation of lipogenic enzymes.

EXPERIMENTAL PROCEDURES

Animals—p53^{+/-}-C57BL/6J (28) and *Lep*^{+*ob*}-C57BL/6J mice were purchased from the Jackson Laboratories (Bar Harbor, Maine). These mice were intercrossed to produce double homozygotes. Genotypes at the p53 loci were determined using the PCR method with primer pairs specific for the wild-type allele of p53 (primers directed against p53 exon 6 and 7; 5'-GGAAATTTGTATCCCGAGTATCTG-3' and 5'-GTCT-TCCAGTGTGATGATGGTAA-3', respectively) and the mutant allele of p53 (primers directed against *neo*; 5'-ATGATTGAACAAGATGGAT-TGC-3' and 5'-TCGGTCTTGACAAAAAGAACC-3'). Genotypes at the leptin loci were determined by a PCR-based restricted fragment length polymorphism analysis as previously described (19). Mice were housed in a temperature-controlled environment with a 12-h light/dark cycle and free access to water and a standard chow diet (Oriental MF, Oriental Yeast, Tokyo, Japan). All experiments were performed with 12-week-old male mice. For fasting and refeeding studies, mice were fasted for 24 h or refed for 12 h after 24-h starvation. For the *ob/ob* × p53^{-/-} experiment, mice were refed for 12 h following a 24-h fast prior to sacrifice. All animals were sacrificed in an early phase of the light cycle.

Nuclear Protein Extraction and Immunoblotting—Nuclear extract protein from white adipose tissue was prepared as described previously (27). Briefly, fresh adipose tissue (~3 g pooled from 3–10 male mice) was rinsed in ice-cold PBS, minced, and homogenized with 10 strokes of a Teflon homogenizer in 15 ml of NDS buffer at 4 °C (10 mM Tris, pH 7.5, 10 mM NaCl, 60 mM KCl, 0.15 mM spermine, 0.5 mM spermidine, 14 mM mercaptoethanol, 0.5 mM EGTA, 2 mM EDTA, 0.5% Nonidet P-40, 1 mM dithiothreitol) supplemented with protease inhibitors (12.5 µg/ml *N*-Acetyl-Leu-Leu-norleucinal-CHO (ALLN, Calbiochem), 2.5 µg/ml leupeptin, 2.5 µg/ml aprotinin, 2.5 µg/ml pepstatin A, 0.1 mM phenylmethylsulfonyl fluoride). Nonidet P-40 concentration was increased to 1% and nuclei were pelleted at 700 × *g* for 10 min, washed once with 25 ml NDS buffer (1% Nonidet P-40), filtered through 70 µm mesh, pelleted at 500 × *g* for 10 min, resuspended in 1 volume of 1% citric acid, lysed by the addition of 2.5 volumes of 0.1 M Tris, 2.5% SDS and 0.1 M dithiothreitol, sonicated briefly, and heated to 90 °C for 5 min. Aliquots of nuclear protein (15 µg) were subjected to SDS/PAGE. p53 was detected using a 1:500 dilution of anti-p53FL (sc-6243, Santa Cruz Biotechnology). The phosphorylation at Ser-15 of p53 was detected using a 1:1000 dilution of anti-p53Ser-15 (9284S, New England Biolabs). To confirm equal loading of nuclear protein, a 1:500 dilution of anti-c-myc (sc-764, Santa Cruz Biotechnology) was also used. Bound antibodies were detected using a horseradish peroxidase-coupled anti-rabbit IgG secondary antibody (Amersham Biosciences) and visualized using ECL Western blotting detection system kit (Amersham Biosciences).

Northern Blotting—Total RNA from epididymal fat pad was extracted using Trizol reagent (Invitrogen), and 10 µg RNA samples equally pooled from each group (*n* = 3–6) were run on a 1% agarose gel containing formaldehyde and transferred to a nylon membrane. For experiments with isolated adipocytes, total RNA from adipocytes isolated by collagenase digestion was prepared as previously described (29). In brief, fresh adipose tissue (~4 g) was minced in 8 ml buffer A (10 mM HEPES at pH 7.4, 130 mM NaCl, 5.2 mM KCl, 1.3 mM KH₂PO₄, 2.7 mM CaCl₂, 1.3 mM MgSO₄, 25 mM NaHCO₃, 3% BSA, 2 mM glucose, 200 nM adenosine) at 37 °C and mixed with equal volume of buffer A containing 4 mg/ml collagenase (type II for adipocyte, Sigma). Following 1-hour digestion with gentle shaking at 37 °C, adipocytes were filtered through 250 µm nylon mesh, washed with 30 ml of buffer A, transferred to another tube, and mixed with 4 volumes of Trizol reagent (Invitrogen). Total RNA sample (10 µg) from one mouse was applied on each lane. The cDNA probes for SREBP-1, fatty acid synthase, ATP citrate lyase, spot 14 and 36B4 (acidic ribosomal phosphoprotein P0) were cloned as described previously (30, 31). The cDNA probes for p53, p21, p27, mdm-2, Bax α, insulin-like growth factor-binding protein-3 (IGFBP-3), glycerol-3-phosphate dehydrogenase (GPDH) and tumor necro-

sis factor α (TNFα) were prepared by cloning RT-PCR products from mouse liver or adipose tissue RNA into pGEM-T easy vectors (Promega). The primers used for PCR were as follows: for p53, 5' primer was 5'-GGAAATTTGTATCCCGAGTATCTG-3' and 3' primer was 5'-GTC-TTCCAGTGTGATGATGGTAA-3'; for p21, 5' primer was 5'-TGTCCA-ATCCTGGTGTATGTC-3' and 3' primer was 5'-TCTCTTGCGAAGAC-CAATCTG-3'; for p27, 5' primer was 5'-GTCAAACGTGAGAGTGTCT-AACG-3' and 3' primer was 5'-GCGAAGAAGAATCTTCTGCA-3'; for mdm-2, 5' primer was 5'-CCAGCCAATGTGCAATAC-3' and 3' primer was 5'-GTGAGCAGGTCAGCTAGTTGAA-3'; for Bax α, 5' primer was 5'-GCTCTGAACAGATCATGAAGACA-3' and 3' primer was 5'-CATGATGGTTCTGATCAGCTC-3'; for IGFBP-3, 5' primer was 5'-CATGCCAAGATGGATGTCATC-3' and 3' primer was 5'-GAGGCA-ATGTACGTCGTCTTTC-3'; for GPDH, 5' primer was 5'-CCATGGCT-GGCAAGAAAGT-3' and 3' primer was 5'-AATCATTTCAGAAATGAG-CTTCAG-3'; for TNFα, 5' primer was 5'-GGTCTGTCCCTTTCACCT-ACTG-3' and 3' primer was 5'-TTGACCTCAGCGTGTGTTG-3'. The probes were labeled with [α-³²P]dCTP using Megaprime DNA labeling system kit (Amersham Biosciences). The membranes were hybridized with the radiolabeled probe in Rapid-hyb Buffer (Amersham Biosciences) at 65 °C with the exception of p53 and TNFα, for which ULTRAhyb hybridization buffer (Ambion) was used at 42 °C. The membranes were washed in 0.1× SSC, 0.1% SDS at 65 °C. Blots were exposed to Kodak XAR-5 film.

Plasmid Constructions—Luciferase gene constructs containing 2.6-kb fragments of the mouse SREBP-1c promoter (pBP1c-Luc) and 0.24-kb fragments of the rat fatty acid synthase promoter (pFAS-Luc) were prepared as described previously (32, 33). Plasmid pGPDH-Luc was constructed by PCR amplification of -489 bp to +23 bp of the mouse glycerol-3-phosphate dehydrogenase (*GPDH*) promoter region (34) and insertion of the PCR products into pGL2-basic vector (Promega). Plasmid p53-Luc, a luciferase reporter plasmid containing 14 tandem copies of p53-binding motif (TGCCTGGACTTGCCTGG) and the luciferase gene was purchased from Stratagene. Mouse p53 expression plasmid driven by the CMV promoter (pCMV-p53) was constructed by inserting a DNA fragment amplified with PCR using first strand cDNA from mouse adipose tissue as a template, two primers (5' primer: 5'-TGGCTGTAGGTAGCGACTACAGTTA-3' and 3' primer: 5'-AG-GCAGTCAGTCTGAGTCAGG-3') and Platinum Pfx DNA polymerase (Invitrogen) into pCMV7 vector (35). All DNA fragments generated with PCR were verified by sequencing.

Transfections and Luciferase Assays—p53-null human osteogenic sarcoma cell line SaOS-2 was obtained from the American Type Culture Collection (ATCC). SaOS-2 cells were cultured in Dulbecco's modified Eagle's medium containing 25 mM glucose, 100 units/ml penicillin, and 100 µg/ml streptomycin sulfate supplemented with 10% fetal calf serum under 5% CO₂ at 37 °C. On day 0, cells were plated on a 12 well plate at 5 × 10⁴ cells/well. On day 3, pCMV-p53 plasmid (1.0 µg), each luciferase reporter plasmid (0.25 µg), and a CMV-β-galactosidase reference plasmid (pCMV-β-gal, Promega, 0.1 µg) were transfected into SaOS-2 cells using FuGENE 6 transfection reagent (Roche Applied Science) according to the manufacturer's protocol. As a control, the same amount of empty vector pCMV7 was transfected instead of pCMV-p53 plasmid. After 48-h incubation, cells were harvested, and the amount of luciferase activity in transfectants was measured by standard kits (Promega).

RESULTS

p53 Activation upon Refeeding in *ob/ob* Adipocytes at the mRNA Level—In our first series of experiments, we demonstrated that *ob/ob* mouse adipose tissue had higher levels of p53 mRNA and protein than did wild type (Fig. 1). p53 is known to be regulated at different levels including through transcription, protein phosphorylation and degradation, and we found that the primary step of p53 activation in *ob/ob* mouse adipose tissue was at mRNA level, although this does not rule out additional post-transcriptional regulations. Consistent with the induced expression, an active form of p53, phosphorylated at Ser-15, was also up-regulated in *ob/ob* adipose tissue (Fig. 1b).

Next we evaluated the mRNA abundance of p53 under various nutritional conditions. To our surprise, the increased expression of p53 in *ob/ob* mouse adipose tissue was limited to a fed state and no difference was observed in a fasted state (Fig. 2a). The mRNA elevation of p53 appeared to be fully induced

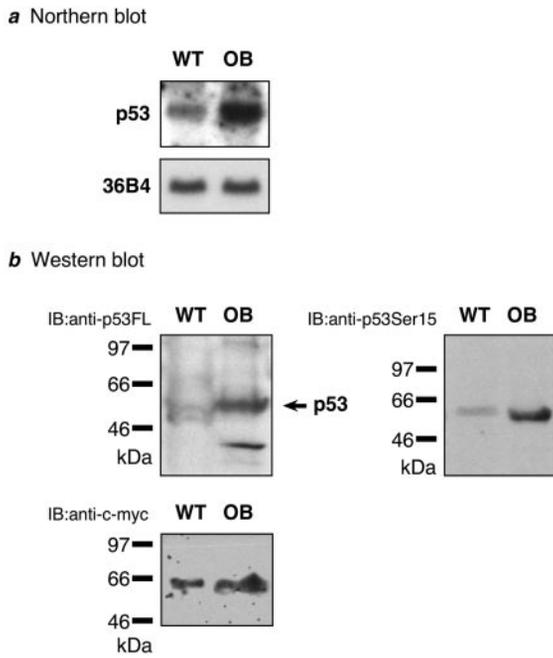


FIG. 1. p53 is increased in *ob/ob* mouse adipose tissue at the mRNA level. *a*, Northern blot and *b*, Western blot analysis of p53 in wild type (*WT*) and *ob/ob* (*OB*) mouse adipose tissue. Mice were sacrificed in refed state. *a*, total RNA (10 μ g) was subjected to Northern blotting, followed by hybridization with the indicated cDNA probes. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading. *b*, immunoblotting using antibody against full-length p53 protein (*left upper panel*) and against phosphorylated Ser-15 (*right upper panel*) is shown. Immunoblotting using antibody against c-Myc (*lower panel*) is shown to indicate equal loading.

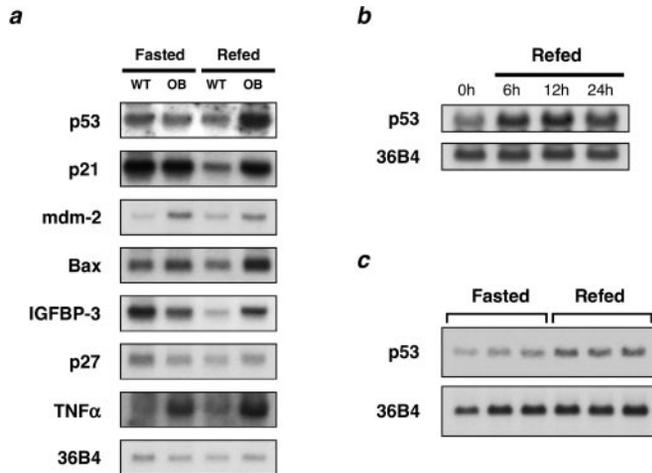


FIG. 2. p53 induction upon refeeding in *ob/ob* mouse adipocytes. *a*, Northern blot analysis of p53 and its downstream genes in adipose tissue from wild type (*WT*) and *ob/ob* (*OB*) mice (six male mice each) is shown. Mice were sacrificed in a fasted (*left two lanes*) or refed state. *b*, time course of p53 induction after refeeding in *ob/ob* mouse adipose tissue. Northern blot analysis of p53 in *ob/ob* mice (three male mice each) refed for the indicated time is shown. *c*, Northern blot analysis of isolated adipocytes from fasted (*left three lanes*) or refed (*right three lanes*) *ob/ob* mice. Total RNA (10 μ g) was subjected to Northern blotting, followed by hybridization with the indicated cDNA probes. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading.

within 6 h after refeeding (Fig. 2*b*). p53 was revealed to be induced mainly in adipocytes when they were isolated from stromal cells by collagenase digestion (Fig. 2*c*), and the residual stromal cells showed far lower levels of p53 expression which did not rise upon refeeding (data not shown).

p53 Activation in ob/ob Adipocytes Is Associated with Induc-

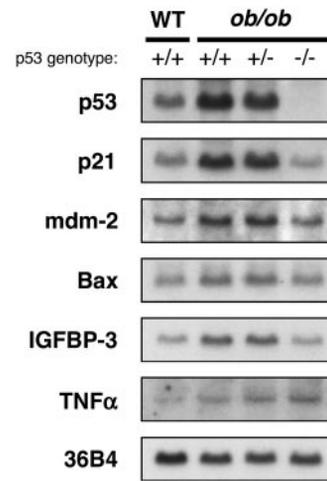


FIG. 3. Effects of p53 absence on the mRNA expression of p53-regulated genes in *ob/ob* mouse adipose tissue. Northern blot analysis of various genes including p53-regulated genes in adipose tissue from refed mice. Lanes (*left to right*) show wild type, *ob/ob*, *ob/ob* \times *p53*^{+/-} and *ob/ob* \times *p53*^{-/-}. Total RNA (10 μ g) pooled equally from six male mice was subjected to Northern blotting, followed by hybridization with the indicated cDNA probes. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading.

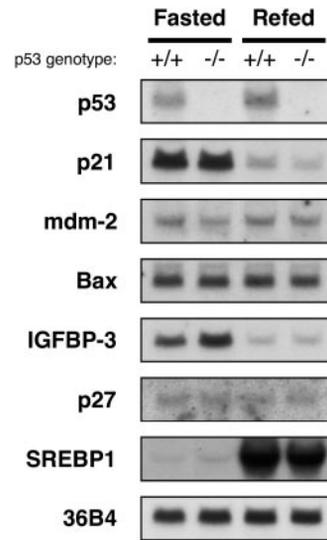


FIG. 4. Up-regulation of p21 by fasting is independent of p53. Northern blot analysis of various genes including p53-regulated genes in adipose tissue from wild type and *p53*^{-/-} mice is shown. Mice were sacrificed in a fasted (*left two lanes*) or refed state. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading.

tion of p53-regulated Genes—To clarify the potential role of p53 in *ob/ob* adipocytes, we further examined the expression profile of p53 downstream genes such as p21, mdm-2, Bax α , and insulin-like growth factor binding protein-3 (IGFBP-3). As shown in Fig. 2*a*, these p53-regulated genes were elevated in adipose tissue of refed *ob/ob* mice. These data indicate that p53 activation causes the up-regulation of its target genes in *ob/ob* adipocytes.

Effects of p53 Absence on the mRNA Expression of p53-regulated Genes in ob/ob Mouse Adipose Tissue—To assess the effects of p53 deficiency in *ob/ob* mice and validate that the elevation of p53 downstream genes are really caused by p53, we intercrossed *ob/ob* and *p53*-null mice, and obtained 6 male mice deficient in both leptin and p53 (*ob/ob* \times *p53*^{-/-}) in the C57BL/6J background. Doubly mutant *ob/ob* \times *p53*^{-/-} mice

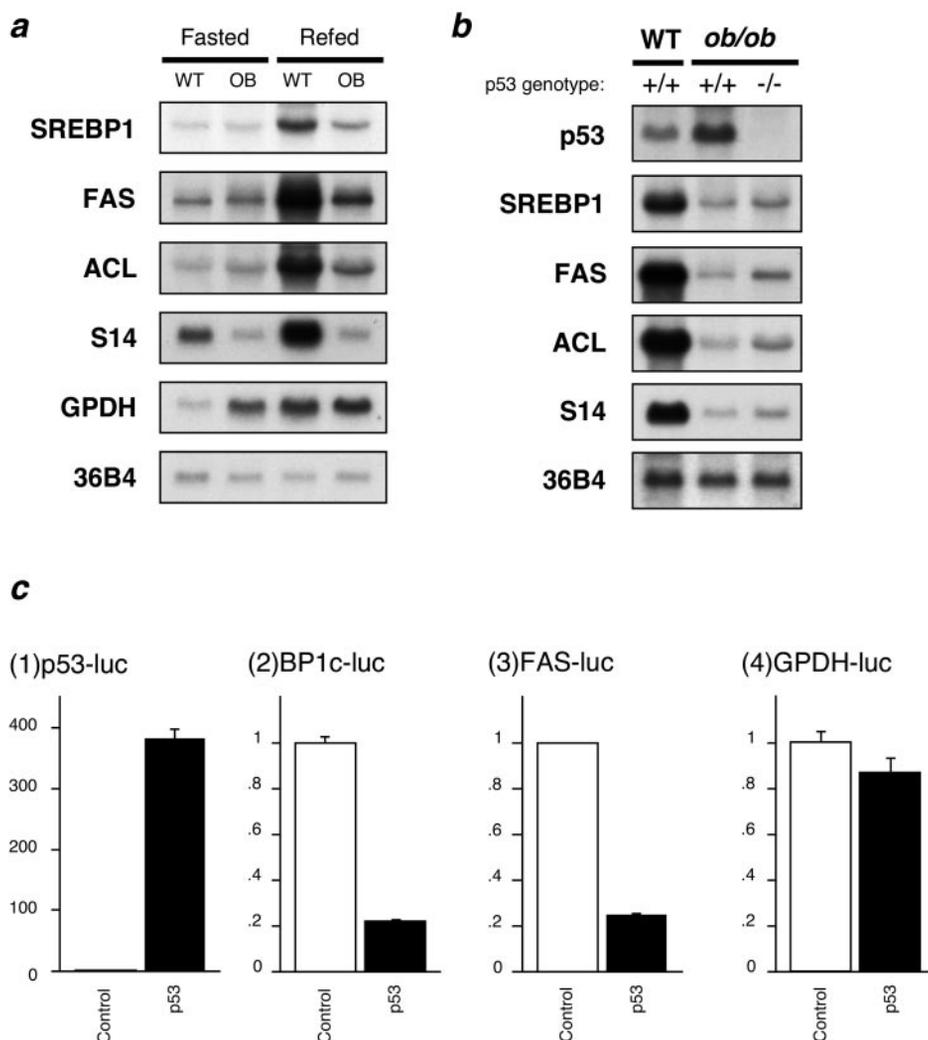


FIG. 5. p53 overexpression suppresses lipogenic gene responses upon refeeding. *a*, defective refeeding responses of lipogenic genes in *ob/ob* mouse adipose tissue. Northern blot analysis of various genes related to lipogenesis in adipose tissue from wild type and *ob/ob* mice is shown. Mice were sacrificed in a fasted (*left two lanes*) or refed state. *b*, partial de-repression of lipogenic genes in *ob/ob* mice lacking p53. Northern blot analysis of lipogenic genes in adipose tissue from refed mice. Lanes (*left to right*) show wild type, *ob/ob* and *ob/ob* × *p53*^{-/-}. FAS, fatty acid synthase; ACL, ATP citrate lyase; S14, Spot 14. *c*, lipogenic gene suppression by p53 overexpression. p53-null SaOS-2 cells were cotransfected with indicated promoter-reporter plasmid and an expression vector, either empty pCMV7 vector (□) or pCMV-p53 expression vector (■). Luciferase activities are indicated as relative values to control. The figure shows one representative data out of four independent experiments. Bars, S.E. BP1c, sterol regulatory element-binding protein-1c.

showed no significant difference in body weight, epididymal fat pad weight, plasma glucose or insulin concentration compared with *ob/ob* mice (data not shown). The Northern blot analysis on these mice in a refed state (Fig. 3) exhibited that p53-regulated genes such as p21, mdm-2, Bax α , and IGFBP-3 in *ob/ob* mice lacking p53 were completely suppressed to the same levels as in wild type. These results established that p53 induction after refeeding caused the elevated expression of its target genes in *ob/ob* adipocytes. Heterozygotes of p53 gene disruption in *ob/ob* mice maintained the similar levels of p53 downstream genes to those of *ob/ob* mice conceivably by compensation by the intact allele.

Up-regulation of p21 and IGFBP-3 by Fasting Is Independent of p53—In our studies of fasted and refed mice, we also found that p21 and IGFBP-3 are elevated when wild-type mice are fasted. To determine whether these changes are ascribable to p53, we analyzed p53^{-/-} mice in fasted or refed conditions. As shown in Fig. 4, the elevation of p21 and IGFBP-3 in a fasted state was also observed in p53^{-/-} mice, and hence, entirely independent of p53.

Defective Refeeding Responses of Lipogenic Genes in *ob/ob* Mice Is Mediated by p53—Lipogenic enzymes such as fatty acid

synthase and ATP citrate lyase are known to be markedly induced in adipose tissue and liver when animals are refed after starvation. In contrast, we have previously reported that the adipose tissue of *ob/ob* mice shows lower levels and defective refeeding responses in the expression of lipogenic enzymes as well as SREBP-1 that regulates their transcription (19). In the current studies we found that the suppression of lipogenic enzymes in *ob/ob* adipose tissue is confined to a subgroup of enzymes such as fatty acid synthase and ATP citrate lyase, whose expression is primarily dominated by SREBP-1 (Fig. 5a). In contrast, glycerol-3-phosphate dehydrogenase, a key enzyme for glycerogenesis and also important for lipogenesis, was not suppressed in refed *ob/ob* mouse adipose tissue. Based on the lack of change in its gene expression by the SREBP-1 overexpression, we assume that glycerol-3-phosphate dehydrogenase is not an SREBP-1 target gene.² These results suggest that p53 suppresses lipogenic gene expression in *ob/ob* adipocytes by the inhibition of SREBP-1 expression.

To test this hypothesis, we evaluated the mRNA expression of lipogenic genes in doubly mutant *ob/ob* × *p53*^{-/-} mice. Northern blot analysis displayed that lipogenic enzymes such as fatty acid synthase and ATP citrate lyase along with SREBP-1 were

moderately elevated in *ob/ob* mice lacking p53, showing that the absence of p53 partially de-suppresses lipogenic gene down-regulation (Fig. 5b). These findings demonstrate that p53 is involved in the suppression of lipogenic genes in *ob/ob* adipocytes. However, the restoration was limited within relatively minor range, suggesting that other factors than p53 are involved in this negative regulation.

Mechanism by Which p53 Suppresses Lipogenic Gene Expression—To explore the mechanism by which p53 suppresses lipogenic gene expression, we performed luciferase reporter assays in cultured cells. We used p53-null cell line SaOS-2 as the transfectant. As shown in Fig. 5c, p53 overexpression suppressed the promoter activity of fatty acid synthase gene as well as that of SREBP-1c gene. In contrast, the promoter activity of glycerol-3-phosphate dehydrogenase gene was not suppressed by p53, which corresponds to the *in vivo* results described above. These findings provide further evidence that p53 suppress lipogenic genes by reducing SREBP-1 expression.

DISCUSSION

Our present studies clearly demonstrate that p53 is induced upon refeeding in *ob/ob* adipocytes, leading to the negative regulation of lipogenic genes. The mechanisms of p53 induction are currently not clear. However, the causes that lead to p53 activation are diverse, ranging from DNA damage to oxidative stress and hypoxia (23). We speculate that *ob/ob* adipocytes receive various stresses that could induce p53 activation. The autocrine excretion of TNF α , the expression of which is increased in *ob/ob* adipocytes (8), could contribute to it. The enlargement of cell volume might cause intracellular hypoxia that could activate p53. Furthermore, in a fed state *ob/ob* mice are markedly hyperglycemic, which also tends to result in oxidative stress through various mechanisms (36–38). Thus, *ob/ob* adipocytes are presumably placed under stressful conditions where p53 can be activated.

Recently it was reported that the phosphatidylinositol 3-kinase-Akt pathway plays an important role in the regulation of p53 via mdm-2 by its enhancement of mdm-2-mediated ubiquitination and degradation of p53 (39). Hence, it is possible that insulin could suppress p53 activation by this pathway. Given that the insulin signaling via phosphatidylinositol 3-kinase and Akt pathway is attenuated in *ob/ob* adipocytes (40, 41) (*i.e.* insulin resistance), this mechanism might be involved in the elevation of p53.

Conversely, it is also possible that the p53 activation could cause insulin resistance and that p53 could be positioned as a modifier of insulin signaling. In addition to our present finding that p53 down-regulates SREBP-1 and thereby lipogenesis, p53 is reported to up-regulate the phosphatase PTEN (42), which opposes phosphatidylinositol 3-kinase and has been implicated to be related to insulin resistance (43, 44). These data suggest that p53 could negatively regulate insulin action in a broad spectrum. The concept that p53 could compete with insulin signaling in nutritional regulation is highly probable considering that insulin ancestrally belongs to a growth factor family closely related to proto-oncogenes, whereas p53 is a tumor suppressor gene.

Obesity is defined as an enlargement of adipose tissue mass and, in case of *ob/ob* mice, is due to both hyperplasia and hypertrophy of adipocytes (4, 5). Therefore, the hyperplastic adipose tissue in obesity could be regarded as a kind of benign tumor. In fact, it has been recently reported that anti-angiogenic agents developed to treat tumors are also effective for obesity (45). It is well known that in many tumors, although mutated, p53 mRNA are expressed at elevated levels (46). The elevation of p53 both in tumors and in *ob/ob* adipose tissue

might result from a common abnormality characteristic of hyperplastic tissue, for instance, low vascularity.

With respect to the consequences of p53 activation in *ob/ob* adipocytes, we are speculating that, besides insulin signaling, p53 in *ob/ob* adipocytes might influence cellular turnover processes. Adipocytes in *ob/ob* mice are reported to be prone to apoptosis (47). Moreover, some markers of immature adipocytes such as adipocyte differentiation-related protein are increased (48). These facts might perhaps imply that the turnover rate of adipocytes is higher and immature cells are relatively increased in obese animals. Thus, although the exact life cycle of adipocytes is not known and precise measurement of their life span needs to be analyzed, p53 might shorten the life span of adipocytes. In keeping with this, it has been recently reported that a transgenic mouse with chronic p53 activation exhibits accelerated aging of organs including adipose tissue (49).

In conclusion, we discovered that p53 is induced upon refeeding in *ob/ob* adipocytes, a mechanism by which lipogenic genes are negatively regulated. This finding might open up a new aspect of this diverse regulator relating cell growth and nutritional regulation.

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