nature medicine

A crucial role for adipose tissue p53 in the regulation of insulin resistance

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Various stimuli, such as telomere dysfunction and oxidative stress, can induce irreversible cell growth arrest, which is termed 'cellular senescence'[1,2]. This response is controlled by tumor suppressor proteins such as p53 and pRb. There is also evidence that senescent cells promote changes related to aging or age-related diseases[3–6]. Here we show that p53 expression in adipose tissue is crucially involved in the development of insulin resistance, which underlies age-related cardiovascular and metabolic disorders. We found that excessive calorie intake led to the accumulation of oxidative stress in the adipose tissue of mice with type 2 diabetes–like disease and promoted senescence-like changes, such as increased activity of senescence-associated β-galactosidase, increased expression of p53 and increased production of proinflammatory cytokines. Inhibition of p53 activity in adipose tissue markedly ameliorated these senescence-like changes, decreased the expression of proinflammatory cytokines and improved insulin resistance in mice with type 2 diabetes–like disease. Conversely, upregulation of p53 in adipose tissue caused an inflammatory response that led to insulin resistance. Adipose tissue from individuals with diabetes also showed senescence-like features. Our results show a previously unappreciated role of adipose tissue p53 expression in the regulation of insulin resistance and suggest that cellular aging signals in adipose tissue could be a new target for the treatment of diabetes.

Cellular senescence was originally defined as the finite replication of human somatic cells in culture. As a consequence of semiconservative DNA replication, the ends of the chromosomes (telomeres) are not duplicated completely, resulting in successive shortening of the telomeres with each cell division[7]. Telomerase is a ribonucleoprotein that adds telomeres to the ends of chromosomes. Telomeres that have shortened beyond a critical threshold, resulting in cell death or senescence, are thought to cause DNA damage that induces cellular senescence. It is now apparent that senescence can be induced by various stresses independently of cell replication, such as chromatin damage related to oxidative stress, and cellular senescence is believed to be a potent anticancer mechanism. Accumulating evidence also suggests a potential relationship between cellular senescence and aging of organisms[8,9].

Aging is known to increase the prevalence of metabolic disorders such as diabetes. Therefore, we hypothesized that cellular aging might influence insulin resistance and accelerate the development of diabetes. To test our hypothesis, we used Ay mice with ectopic expression of agouti peptide, which leads to perturbation of the central melanocortin pathway and thereby induces excessive calorie intake, resulting in obesity and diabetes. It has been reported that production of reactive oxygen species (ROS) is selectively increased in the adipose tissue of obese mice and that increased oxidative stress in fat is a key mechanism underlying the occurrence of insulin resistance related to obesity[10]. Consistent with previous studies, Ay mice on a normal diet for 20 weeks showed higher adipose tissue amounts of ROS compared with wild-type mice on the same diet (**Supplementary Fig. 1a**). Because increased stress due to ROS can induce DNA damage and subsequent activation of p53, leading to telomere-independent senescence[3,4], we tested whether adipose tissue of Ay mice shows a senescence-like phenotype. The adipose tissue of these mice showed senescence-like changes, including enhanced activity of senescence-associated β-galactosidase (SA-β-gal; **Fig. 1a**). Ay mice also showed higher adipose tissue amounts of p53 on the protein level and cyclin-dependent kinase inhibitor-1A (*Cdkn1a*) expression on the mRNA level compared to wild-type mice (**Fig. 1b**,**c**), suggesting excessive caloric intake can induce senescence-like changes in adipose tissue.

It has been reported that increased secretion of proinflammatory cytokines by adipose tissue exacerbates insulin resistance in people with metabolic disorders[11–13]. Senescent cells are known to secrete molecules that can alter the local microenvironment, such as proinflammatory cytokines[3,5]. We therefore speculated that senescence-like changes might be associated with increased expression of proinflammatory cytokines by adipose tissue that could induce insulin resistance. Consistent with this concept, expression of proinflammatory cytokines such as tumor necrosis factor-α (*Tnf*) and chemokine (C-C motif) ligand-2 (*Ccl2*), also known as monocyte chemoattractant protein-1, was upregulated in association with an increase

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Received 25 March; accepted 29 June; published online 30 August 2009; doi:10.1038/nm.2014

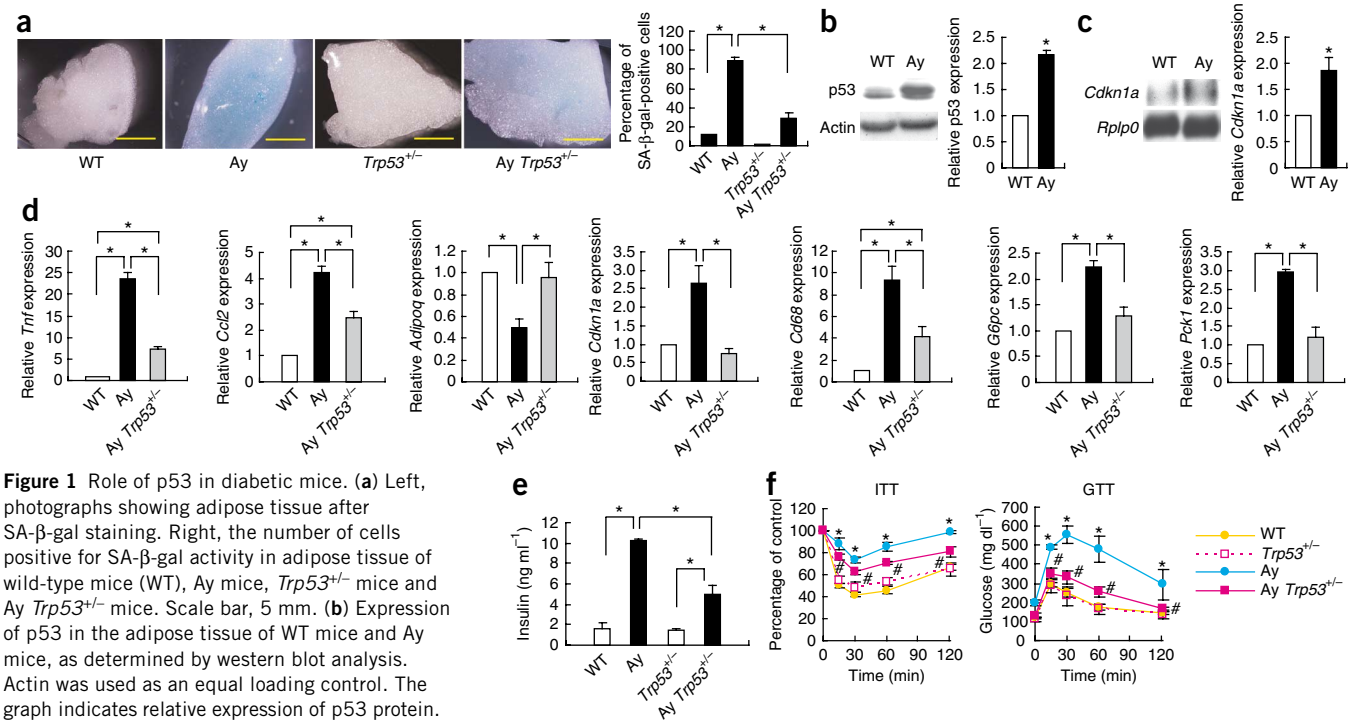

Figure 1 Role of p53 in diabetic mice. (**a**) Left, photographs showing adipose tissue after SA-β-gal staining. Right, the number of cells positive for SA-β-gal activity in adipose tissue of wild-type mice (WT), Ay mice, *Trp53*$^{+/-}$ mice and Ay *Trp53*$^{+/-}$ mice. Scale bar, 5 mm. (**b**) Expression of p53 in the adipose tissue of WT mice and Ay mice, as determined by western blot analysis. Actin was used as an equal loading control. The graph indicates relative expression of p53 protein. (**c**) Expression of *Cdkn1a* in the adipose tissue WT mice and Ay mice, as determined by northern blot analysis. Ribosomal protein, large, P0 (Rplp0) was used as an equal loading control. The graph indicates relative expression of *Cdkn1a* mRNA. (**d**) Real-time PCR assessing the expression of cytokines, *Cdkn1a* and *Cd68* in adipose tissue and the expression of *G6pc* (encoding glucose-6-phosphatase) and *Pck1* (encoding phosphoenolpyruvate carboxykinase) in the livers of WT mice, Ay mice and Ay *Trp53*$^{+/-}$ mice. (**e**) Plasma insulin concentrations in WT mice, Ay mice, *Trp53*$^{+/-}$ mice and Ay *Trp53*$^{+/-}$ mice. $*P < 0.05$; $n = 4$–6 for **a** and **d**; $n = 3$ for **b**; $n = 4$ for **c** and **e**. (**f**) Insulin tolerance test (ITT) and glucose tolerance test (GTT) in WT mice, Ay mice, *Trp53*$^{+/-}$ mice and Ay *Trp53*$^{+/-}$ mice. $*P < 0.05$ versus WT; $\#P < 0.05$ versus Ay; $n = 7$. Data are shown as the means ± s.e.m.

of macrophage marker expression, whereas expression of anti-inflammatory cytokines (including adiponectin, (*Adipoq*)) was downregulated in the adipose tissue of Ay mice (**Fig. 1d**). We detected upregulation of inflammatory cytokines, as well as of p53 protein and *Cdkn1a* expression, in both the stromal vascular fraction (macrophage-rich fraction) and the adipose-rich fraction (**Supplementary Fig. 1b**), suggesting that senescence of both macrophages and adipocytes causes an inflammatory response that leads to insulin resistance.

We next investigated whether inhibition of p53 could reverse insulin resistance and glucose intolerance in Ay mice. The number of SA-β-gal–positive cells and the expression of *Cdkn1a* were significantly lower in adipose tissue from Ay *Trp53*$^{+/-}$ mice than in tissue from Ay *Trp53*$^{+/+}$ mice (**Fig. 1a**,**d**), whereas there was no significant difference in food intake between the two groups (**Supplementary Fig. 1c**). The fat weight of Ay *Trp53*$^{+/-}$ mice was slightly lower than that of Ay *Trp53*$^{+/+}$ mice (**Supplementary Fig. 2a**). Reduced activation of p53 led to lower plasma insulin concentrations in Ay mice and also to normalization of cytokine and macrophage marker expression by adipose tissue (**Fig. 1d**,**e**). Hepatic expression of gluconeogenic enzymes was also lower in Ay *Trp53*$^{+/-}$ mice (**Fig. 1d**). Consistent with these changes, Ay *Trp53*$^{+/-}$ mice showed significantly better insulin sensitivity and glucose tolerance compared with Ay *Trp53*$^{+/+}$ mice as determined by insulin and glucose tolerance tests (**Fig. 1f**).

Because Ay *Trp53*$^{+/-}$ mice have p53 haploinsufficiency throughout the whole body, improvement of insulin resistance might be attributable to inhibition of p53 activity in other tissues aside from the white adipose tissue. To investigate the role of adipose tissue p53 in the regulation of insulin resistance, we generated mice with adipocyte-specific p53 deficiency (adipo-p53–deficient mice), using transgenic mice that express Cre recombinase under control of the mouse fatty acid–binding protein-4 (*Fabp4*) promoter, and fed these mice a high-fat, high-sucrose (HF-HS) diet for 4 months. Expression of p53 protein and *Cdkn1a* mRNA in adipose tissue was significantly upregulated in littermate control mice on the HF-HS diet, whereas this increase was significantly attenuated in adipo-p53–deficient mice (*Trp53*$^{loxP/loxP}$ Fabp4-Cre) receiving the same diet (**Fig. 2a**,**b** and **Supplementary Fig. 1d**). These mice had a slightly smaller increase of fat weight (**Supplementary Fig. 2b**) and normalized expression of adipokines and hepatic gluconeogenic enzymes (**Fig. 2b**), whereas hepatic p53 protein expression was unchanged (**Fig. 2a**). Insulin-induced phosphorylation of Akt was also restored in adipo-p53–deficient mice (**Supplementary Fig. 1e**). Consequently, insulin resistance induced by the HF-HS diet was lower in mice with adipocyte-specific ablation of p53 compared to control mice (**Fig. 2c**), indicating that p53 expression in adipose tissue has a crucial role in the development of insulin resistance.

It has been reported that Fabp4 is expressed in hematopoietic cells and has considerable influence on various biological responses[14,15]. To examine the possible involvement of p53 in hematopoietic cells, we transplanted wild-type bone marrow cells into adipo-p53–deficient mice or littermate control mice and induced dietary obesity in these mice. Adipo-p53–deficient mice transplanted with wild-type bone marrow cells showed better glucose tolerance than littermate control mice transplanted with wild-type marrow cells, but their glucose tolerance was impaired compared with that of adipo-p53–deficient mice without bone marrow transplantation (**Supplementary Fig. 1f**). In adipose-p53–deficient mice, expression of p53 protein and *Cdkn1a* was considerably lower in both the stromal vascular fraction and the

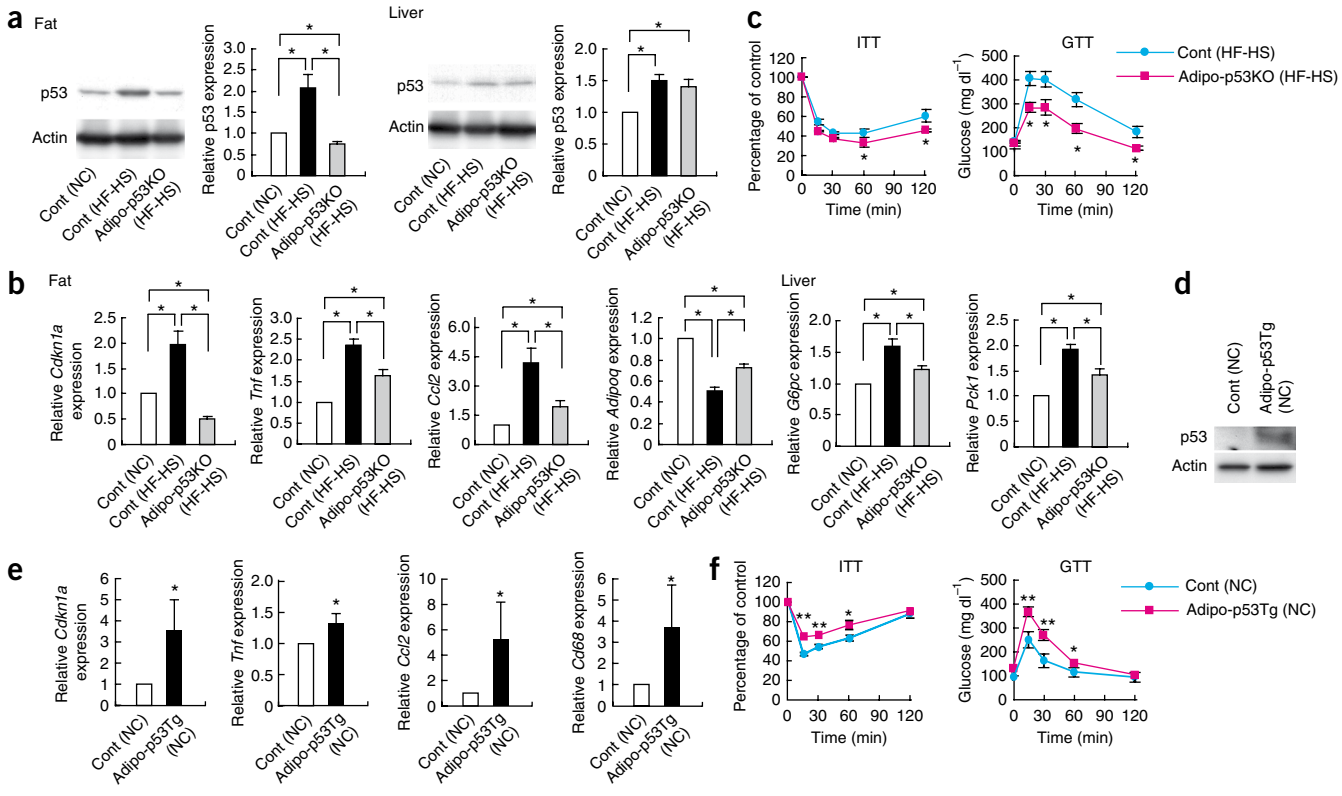

Figure 2 Adipose tissue p53 expression contributes to insulin resistance in mice with dietary obesity. (**a**) Western blot analysis for p53 in the fat and liver of littermate controls (Cont) on a normal diet (normal chow, NC), littermate controls (Cont) on an HF-HS diet (HF-HS), and adipo-p53–deficient mice (Adipo-p53KO) on an HF-HS diet (HF-HS). The graph indicates relative expression of p53 protein. (**b**) Real-time PCR assessing the expression of *Cdkn1a*, *Tnf*, *Ccl2* and *Adipoq* in adipose tissue and *G6pc* and *Pck1* in liver of the same mice as in **a**. $*P < 0.05$; $n = 5$ mice for **a** and **b**. (**c**) ITT and GTT in Adipo-p53KO mice and littermate controls (Cont) after 4 months on a HF-HS diet or a normal diet. $*P < 0.05$ versus control (HF-HS); $n = 8$. (**d**) Western blot analysis for p53 in adipose tissue of littermate controls (Cont) and adipo-p53–transgenic (Adipo-p53Tg) mice on a normal diet (NC). (**e**) Real-time PCR assessing the expression of *Cdkn1a*, *Tnf*, *Ccl2* and *Cd68* in adipose tissue of the same mice as in **d**. $*P < 0.05$; $n = 5$. (**f**) ITT and GTT in Adipo-p53Tg mice and littermate controls (Cont) on a normal diet (NC). $*P < 0.05$, $**P < 0.01$ versus control; $n = 8$. Data are shown as the means ± s.e.m.

adipose-rich fraction compared with the levels seen in littermate control mice (**Supplementary Fig. 1d**). These results suggested that both macrophages and adipocytes contribute to the senescence-like changes of adipose tissues in mice with dietary obesity and that upregulation of p53 expression in adipose tissue has a pathological role in producing insulin resistance.

To further investigate the role of adipose tissue p53, we established transgenic mice that showed an increase of p53 protein and *Cdkn1a* mRNA expression in adipose tissue (**Fig. 2d**,**e**). Consistent with the results found in adipose-p53–deficient mice, these transgenic mice on a normal diet showed higher expression of proinflammatory cytokines and a macrophage marker (**Fig. 2e**), which was associated with impairment of insulin sensitivity and glucose tolerance (**Fig. 2f**), providing further evidence for a major role for adipose tissue p53 in insulin resistance.

Insulin resistance has been reported to correlate with enhanced telomere shortening in young adults[16], whereas aging is known to accelerate telomere dysfunction in various human tissues[1,5]. It is well accepted that dysfunctional (that is, critically short) telomeres resemble damaged DNA and thus trigger a p53-dependent response[17,18]. To investigate a potential relationship between telomere-dependent p53 activation and insulin resistance, we used telomerase reverse transcriptase (*Tert*)-deficient mice. These mice have a normal phenotype in the first generation (G1), presumably because mice possess very long telomeres[19,20]. However, their telomeres become shorter with successive generations, and the mice eventually become infertile by the fourth to sixth generation (G4–G6), owing to impairment of the reproductive system[20]. We fed an HF-HS diet to G4 mice for 8 weeks (from 4 to 12 weeks of age) and examined the effects of cellular aging on glucose metabolism. Although the insulin sensitivity and glucose tolerance of G4 mice were similar to those of wild-type mice on a normal diet, insulin resistance and glucose intolerance became more prominent in G4 mice than in wild-type mice after feeding with the HF-HS diet (**Fig. 3a**). There were no significant differences in weight gain, food intake and oxygen consumption between the two groups (**Fig. 3b**). Expression of proinflammatory cytokines such as *Tnf* and *Ccl2* was increased in the adipose tissue of G4 mice on the HF-HS diet, and this increase was evident in mice with shorter telomeres in adipose cells (**Fig. 3c**,**d** and **Supplementary Fig. 3a**). Shorter telomeres also promoted the infiltration of macrophages into adipose tissue (**Fig. 3c** and **Supplementary Figs. 2c** and **3b**). Expression of hepatic gluconeogenic enzymes was upregulated in G4 mice (**Fig. 3c**). Insulin-induced phosphorylation of Akt was markedly lower in the liver of G4 mice compared to wild-type mice, and in skeletal muscle to a lesser extent (**Supplementary Fig. 3c**). The adipose tissue of G4 mice on the HF-HS diet showed senescence-like changes, including

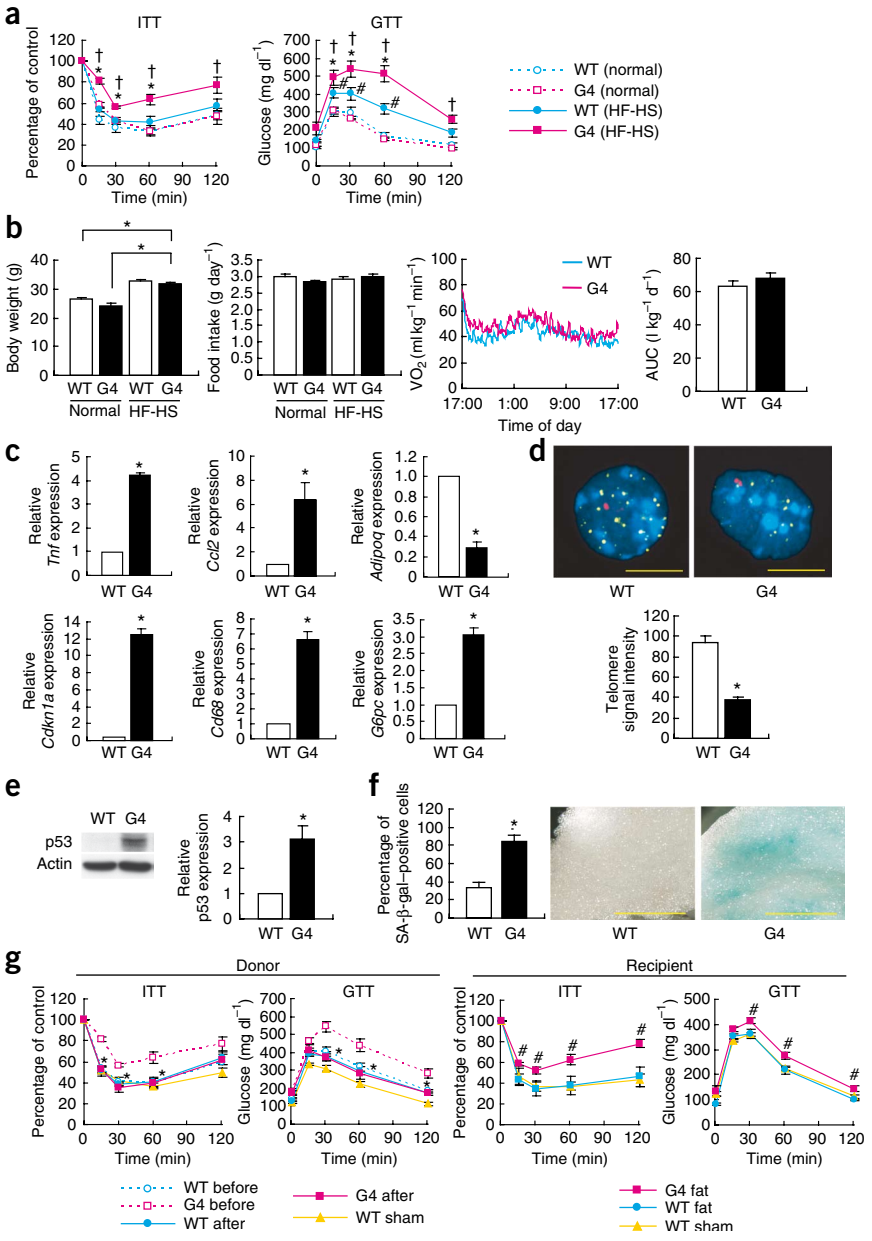

Figure 3 Adipose tissue p53 expression and insulin resistance in G4 mice. (**a**) ITT and GTT after 8 weeks on a HF-HS diet or a normal diet (normal) in G4 and WT mice. $*P < 0.05$ versus WT (HF-HS); $\#P < 0.05$ versus WT (normal); $\dagger P < 0.05$ versus G4 (normal); $n = 7$. (**b**) Body weight, food intake and oxygen consumption (VO_2) in WT and G4 mice. AUC, area under the curve. (**c**) Real-time PCR analysis of the expression of cytokines, *Cdkn1a* and *Cd68* in adipose tissue and the expression of *G6pc* in the livers of WT mice and G4 mice. All mice were fed on the HF-HS diet. (**d**) Top, telomeric fluorescence (yellow) *in situ* hybridization of adipocytes from WT and G4 mice. The signal intensity of the X chromosome (red) was used as an internal control. Bottom, estimation of the length of telomeres in adipose cells by quantification of telomeric fluorescence *in situ* hybridization images. Representative of 30 nuclei (images) for each genotype. Scale bar, 10 μm. (**e**) Expression of p53 in the adipose tissue of WT mice and G4 mice on the HF-HS diet, as determined by western blot analysis. The graph indicates relative expression of p53 protein. (**f**) The number of cells positive for SA-β-gal activity in the adipose tissue of WT mice and G4 mice. Photographs show adipose tissue after SA-β-gal staining. Scale bar, 5 mm. $*P < 0.05$; $n = 6$ for **b**; $n = 5$ for **c**; $n = 30$ nuclei for **d**; $n = 3$ for **e** and **f**. (**g**) Left, ITT and GTT in WT and G4 donor mice before and after fat pad removal. Right, ITT and GTT in recipients of fat pads (1 g) from WT mice (WT fat) or G4 mice (G4 fat) and in sham-operated WT mice (WT sham). $*P < 0.05$ versus G4 before, $\#P < 0.05$ versus WT fat; $n = 6$. Data are shown as the means ± s.e.m.

increased expression of *Cdkn1a* mRNA and p53 protein, as well as enhanced activity of SA-β-gal (**Fig. 3c**,**e**,**f** and **Supplementary Fig. 3d**). These results suggest that telomere-dependent senescence of adipose tissue can also promote an inflammatory response, thereby leading to insulin resistance.

To investigate the influence of adipose tissue senescence on the insulin resistance of G4 mice receiving a HF-HS diet, we transplanted epididymal fat pads subcutaneously into wild-type mice and examined the changes of insulin sensitivity and glucose tolerance in the donor and recipient mice. Two weeks after fat pad removal, insulin resistance and glucose intolerance were both markedly improved in G4 mice on the HF-HS diet (**Fig. 3g** and **Supplementary Fig. 2d**). The insulin level of donor G4 mice was also normalized by 2 weeks after fat pad removal (**Supplementary Fig. 3e**). Conversely, implantation of adipose tissue from G4 mice on this diet significantly impaired the insulin sensitivity and glucose tolerance of wild-type recipient mice, whereas implantation of adipose tissue from other wild-type mice fed the same diet had no effect (**Fig. 3g**). Implantation of adipose tissue from G4 mice also lowered insulin-induced phosphorylation of Akt in the liver (**Supplementary Fig. 3f**). Histological examination showed that the implanted adipose tissue was viable and vascularized (**Supplementary Fig. 3g**). Moreover, implantation of fat pads collected from G4 $Trp53^{+/-}$ mice into wild-type mice had less influence on the insulin resistance and glucose tolerance of the recipients (**Supplementary Fig. 3h**) compared with fat pads from G4 $Trp53^{+/+}$ mice. These results indicate that telomere-dependent p53 activation in adipose tissue also leads to insulin resistance.

We noted that expression of histone γ-H2AX, a marker of double-stranded DNA breaks, was increased in the adipose tissue of Ay mice as well as G4 mice (**Fig. 4a**), suggesting a potential role of the ROS-induced DNA damage pathway in the development of type 2 diabetes. To further investigate the relationship between ROS-induced DNA damage and diabetes, we examined the effect of oxidative stress on the expression of inflammatory cytokines in primary cultures of human preadipocytes. Treatment with hydrogen peroxide led to a marked increase of p53 protein expression (**Fig. 4b**). Hydrogen peroxide treatment significantly upregulated expression of *TNF* and *CCL2*, whereas this upregulation was inhibited by p53 knockdown (**Fig. 4c**). This treatment also increased the activity of nuclear factor-κB (NF-κB);

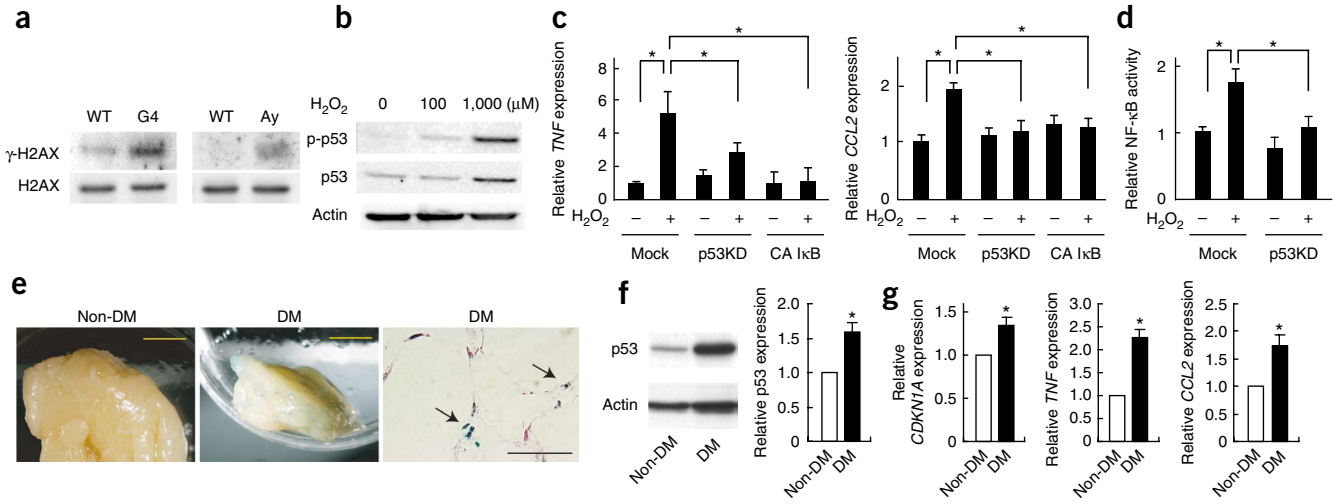

Figure 4 Senescence-like features of adipose tissue from subjects with diabetes. (**a**) Western blot analysis of γ-H2AX expression in adipose tissue of WT mice and G4 mice on a HF-HS diet and WT mice and Ay mice on a normal diet. (**b**) Effect of hydrogen peroxide (H_2O_2) on p53 expression in human preadipocytes by western blot analysis. p-p53, phosphorylated p53. (**c**) Hydrogen peroxide–induced expression of *TNF* and *CCL2* in human preadipocytes transfected with siRNA targeting p53 (p53KD) or the vector encoding constitutively active inhibitor of κB (CA IκB). (**d**) Effect of p53 knockdown (p53KD) on hydrogen peroxide–induced activation of NF-κB. (**e**) Adipose tissue from subjects without diabetes (non-DM) or subjects with diabetes (DM) after SA-β-gal staining. Scale bar, 10 mm. The photograph on the right shows adipose tissue obtained from a subject with diabetes (DM). Arrows indicate SA-β-gal–positive cells. Scale bar, 50 μm. (**f**,**g**) Expression of p53, *CDKN1A* and cytokines in adipose tissue obtained from subjects without diabetes or subjects with diabetes, as determined by western blot analysis (**f**) or real-time PCR (**g**). The graphs indicate relative expression of p53 protein (**f**) and relative mRNA levels of *CDKNA1*, *TNF* and *CCL2* (**g**). $^*P < 0.05$; $n = 5$ for **c**, **d**, **f** and **g**. Data are shown as the means ± s.e.m.

Fig. 4d), a key transcription factor that regulates the induction of cytokines, including *TNF* and *CCL2*, whereas inhibition of NF-κB activation suppressed oxidative stress–induced upregulation of these cytokines (**Fig. 4c**). In agreement with previous reports that induction of p53 causes activation of NF-κB[21,22], we found that p53 deficiency led to a decrease in oxidative stress–induced NF-κB activation (**Fig. 4d**), indicating that ROS-induced p53 activation causes NF-κB–dependent induction of inflammatory cytokines and thus accelerates the development of diabetes.

To determine whether or not senescence-like changes occur in human adipose tissue, we examined visceral fat obtained from subjects undergoing abdominal surgery for primary gastric cancer or colon cancer. Adipose tissue from subjects with diabetes showed increased SA-β-gal activity and higher levels of p53 protein and *CDKN1A* mRNA expression compared with tissue from nondiabetic subjects (**Fig. 4e–g**). Moreover, expression of inflammatory cytokines was significantly increased in diabetic adipose tissue (**Fig. 4g**), suggesting that aging of fat cells has a major role in human diabetes.

Recent studies have shown that longevity signals generated in adipose tissue are crucial in regulating the lifespan of various species, ranging from worms to mice, and suggested that aging is non–cell-autonomously regulated by adipose tissue[23–26]. Consistent with these reports, subcutaneous implantation of senescent adipose tissue from G4 mice accelerates the senescence of epididymal fat in wild-type recipients (T.M., unpublished data). Senescence of adipose tissue may increase the local production of proinflammatory molecules, and it also promotes systemic inflammation and insulin resistance via non–cell-autonomous mechanisms. In contrast, low circulating insulin concentrations are generally associated with longevity, and the activation of longevity signals in adipose tissue has been reported to lower the circulating insulin level and extend the lifespan[27,28]. We found that inhibition of p53 activity in adipose tissue improved insulin resistance and decreased the plasma insulin level. Thus, p53 activation in adipose tissue may be a proaging signal with a negative influence on longevity, whereas inhibition of cellular aging may become a new strategy for the treatment of diabetes as well as aging and its associated diseases.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemedicine/.

Note: Supplementary information is available on the Nature Medicine website.

ACKNOWLEDGMENTS

We thank H. Karagiri for discussion, W.C. Greene (University of California) and T. Fujita (The Tokyo Metropolitan Institute of Medical Science) for expression vector encoding constitutively active IκB and p55-A2-Luc (luciferase reporter vector containing the κB binding sites), respectively, A. Berns (The Netherlands Cancer Institute) for floxed *Trp53* mice, and E. Fujita, Y. Ishiyama, R. Kobayashi and Y. Ishikawa for their excellent technical assistance. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture and Health and Labor Sciences Research Grants (to I.K.); a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan; and grants from the Suzuken Memorial Foundation, the Japan Diabetes Foundation, the Ichiro Kanehara Foundation, the Tokyo Biochemical Research Foundation, the Takeda Science Foundation, the Cell Science Research Foundation and the Japan Foundation of Applied Enzymology (to T.M.).

AUTHOR CONTRIBUTIONS

T.M. designed and conducted experiments and wrote the manuscript, M.O., I.S., T.K., M.Y., T.I., A. Nojima and Y.O. conducted experiments, A. Nabetani performed telomere analysis, H.M. performed the human studies, F.I. generated telomerase-deficient mice and I.K. evaluated the results, supervised this study and wrote the manuscript.

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ONLINE METHODS

Animal models. The study protocol was approved by the Chiba University Institutional Animal Care and Use Committee. The creation of mice deficient in *Tert* has been described previously[29]. We backcrossed heterozygous mice with wild-type C57BL/6 mice (SLC) for six generations and intercrossed them to produce G1 $Tert^{-/-}$ mice. Mating of G1 $Tert^{-/-}$ mice with each other generated G2 mice, after which we produced $Tert^{-/-}$ mice up to the fourth generation (G4). We purchased p53-deficient mice (with a C57BL/6 background) from Jackson Laboratories and mated them with $Tert^{+/-}$ mice to generate double heterozygotes ($Tert^{+/-}$ $Trp53^{+/-}$). We intercrossed these mice to generate G1 $Tert^{-/-}$ $Trp53^{+/-}$ mice. We mated G1 $Tert^{-/-}$ $Trp53^{+/-}$ mice with other G1 mice to produce G2 $Tert^{-/-}$ $Trp53^{+/-}$ mice, after which we repeated this mating strategy to generate G4 $Tert^{-/-}$ $Trp53^{+/-}$ mice. We fed the mice a HF-HS diet (Oriental Yeast)[30] or normal chow from 4 to 12 weeks of age before we performed metabolic analyses. We purchased Ay mice (with a C57BL/6 background) from Jackson Laboratories and mated them with $Trp53^{+/-}$ mice to generate $Trp53^{+/+}$, Ay $Trp53^{+/+}$, $Trp53^{+/-}$ and Ay $Trp53^{+/-}$ mice. We fed these mice normal chow and analyzed them at 20 weeks of age. We purchased mice that express Cre recombinase in adipocytes (Fabp4-Cre) from Jackson Laboratories. We then crossed Fabp4-Cre mice (with a C57BL/6 background) with mice that carry floxed *Trp53* alleles (with a C57BL/6 background)[31] to generate adipocyte-specific p53-knockout mice. We fed these mice a HF-HS diet or normal chow for 4 months before we performed metabolic analyses. Littermate controls had the genotype $Cre^{-}Trp53^{loxP/-}$ or $Cre^{-}Trp53^{loxP/loxP}$. We also generated transgenic mice (with a C57BL/6 background) that carry the *loxP-LacZ-loxP* cassette flanked by the *TP53* complementary DNA fragment under the control of the cytomegalovirus enhancer–chicken actin promoter. Expression of transgene-derived *TP53* was prevented by the *loxP-LacZ-loxP* cassette. When we bred these transgenic mice with Fabp4-Cre mice, the floxed *LacZ* cassette was excised in the resulting offspring ($Cre^{+}LacZ\text{-}TP53^{+}$), and we observed upregulation of p53 expression in adipose tissue (adipo-p53–transgenic mice). We fed these mice normal chow and analyzed them at 10–12 weeks of age. Littermate controls had the genotype $Cre^{-}LacZ\text{-}TP53^{+}$.

Cell culture. We purchased human preadipocytes from Sanko, and we cultured them according to the manufacturer's instructions.

Western blot analysis. We resolved whole-cell lysates (30–50 μg) by SDS PAGE. We transferred the proteins onto a polyvinylidene difluoride (PVDF) membrane (Millipore) incubated them with the primary antibody (**Supplementary Methods**), followed by incubation with rabbit IgG–specific horseradish peroxidase–conjugated antibody (111-035-003) or mouse IgG–specific horseradish peroxidase–conjugated antibody (115-035-003; Jackson). We detected specific proteins by enhanced chemiluminescence (Amersham).

Human subjects. The ethical committee of Chiba University Graduate School of Medicine reviewed and approved the study protocol. We enrolled 10 subjects (56–68 years old; six males and four females) who were admitted to Chiba University Hospital and underwent surgery for primary gastric or colon cancer. We obtained informed consent from all subjects before inclusion in the study.

Statistical analyses. Data are shown as the means ± s.e.m. We examined differences between groups by Student's *t* test or analysis of variance followed by Bonferroni's correction for comparison of means. For all analyses, we considered $P < 0.05$ as statistically significant.

Additional methods. Detailed methodology is described in the **Supplementary Methods**.

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doi:10.1038/nm.2014