Dear Editor,

The p53 tumor suppressor maintains the normal cell growth and genomic stability by launching cell cycle arrest, DNA repair, or apoptosis in response to DNA damage or other forms of cellular stress. Recent studies also suggest that p53 is capable of much broader cellular functions, including the regulation of energy metabolism and autophagy. However, the role of p53 in regulating lipid metabolism is less well understood. Here we report a novel function of p53 in regulating lipid metabolism. Loss of p53 leads to lipid accumulation in both mouse embryonic fibroblast (MEF) cells and mouse liver. Upon high-fat diet (HFD) treatment, p53 knockout mice exhibit marked obesity and hepatic lipid accumulation. Mechanistically, p53 regulates lipid metabolism through transcriptionally regulating aromatase, a key enzyme that converts androgens to estrogens. The importance of aromatase in mediating p53's function in regulating lipid metabolism is revealed by the observation that transgenic expression of aromatase almost completely reverses the promoting effect of p53 deficiency on lipid accumulation in mouse liver.

Our most recent data showed that p53 regulates lipid accumulation by directly binding to G6PD and thus inhibiting pentose phosphate pathway (PPP) (Jiang et al., 2011). To further thoroughly investigate the role of p53 in modulating lipid metabolism, we first compared the lipid content in p53+/+ and p53−/− MEF cells treated with or without oleic acids, a well-known stimulator of triglyceride (TG) synthesis. The p53−/− MEF cells exhibit elevated lipid levels compared with p53+/+ MEF cells as evaluated by Oil Red O (ORO) staining and by BODIPY staining (Supplementary Figure S1A and B), which was consistent with the previous study (Molchadsky et al., 2008). We next evaluated the effect of p53 on the lipid droplet formation in the liver. The liver of p53−/− mice was shown to have a larger number of lipid droplets compared with that of p53+/+ mice (Supplementary Figure S1C). Consistent with this, primary hepatocytes isolated from p53−/− mice showed enhanced lipid levels (Supplementary Figure S1D). Also, the knockdown of p53 strongly increased lipid levels in MEF cells (Supplementary Figure S1E). These results demonstrate that the loss of p53 increases lipid accumulation and suggest the physiological function of p53 in regulating lipid metabolism.

We next examined whether p53 regulates HFD-induced obesity and hepatic steatosis. When fed with normal diets, p53−/− and p53+//+ mice showed no obvious difference in either body mass or abdominal fat accumulation. However, HFD treatment resulted in a substantial increase in the body mass of p53−/− mice compared with p53+/+ mice (Figure 1A and B). Livers from p53−/− mice were generally larger and paler in color than those from p53+/+ littermates after HFD treatment (Figure 1B). HFD treatment also led to an increased accumulation of epididymal white adipose tissue in p53−/− mice compared with p53+/+ mice (Figure 1B). Hematoxylin and eosin staining showed that the liver from p53−/− mice had a greater accumulation of lipid droplets than that from p53 wild-type mice, especially under the HFD treatment condition (Supplementary Figure S2A). Consistently, immunohistochemical staining showed that the lack of p53 resulted in a marked increase in the levels of adipose differentiation related protein (Supplementary Figure S2B), a lipid droplet marker which coats cytoplasmic lipid droplets. Moreover, adipocytes from p53−/− mice were bigger than those from p53 wild-type mice in response to HFD treatment (Supplementary Figure S2C). Altogether, these results suggest that p53 depletion promotes HFD-induced obesity and liver steatosis in male mice.

Interesting enough, after evaluation of the serum levels of testosterone (T) in p53+/+ and p53−/− mice, we surprisingly found that p53−/− mice produced dramatically more testosterone than p53+/+ mice upon HFD treatment, while the serum testosterone levels were not significantly different between these two groups of mice under normal diet condition (Supplementary Figure S2D). In the same experiments, the levels of 17β-oestradiol (E2) were comparatively low in p53−/− and p53+/+ male mice under both normal diet and HFD conditions (Supplementary Figure S2E). As a result, the ratio of T/E2 is significantly higher in p53−/− mice under HFD treatment conditions (Supplementary Figure S2F).

It has been shown that inactivation of aromatase leads to enhanced levels of testosterone and lipid accumulation in the liver (Jones et al., 2000). The observation of elevated levels of testosterone and lipid accumulation in p53−/− mice upon HFD treatment led us to explore the possibility that the levels of aromatase could be decreased in p53−/− mice, thus leading to the inhibition of aromatase enzyme activity. After thorough inspection, one putative p53-binding element was found within intron 1 of aromatase gene (Figure 1C). The subsequent chromatin immunoprecipitation (ChIP) assays showed the specific binding of p53 to the chromatin fragments containing the putative p53-binding element (Figure 1C). In addition, pGL3 luciferase reporter plasmid containing the putative p53-binding element, but not the mutant plasmid, showed a p53-responsive transcriptional activity (Supplementary Figure S3A). The lack of p53 resulted in substantially lower levels of both aromatase mRNA and protein expression (Supplementary Figure S3B). Furthermore, real-time RT-PCR analysis showed that various tissues from p53−/− mice exhibited substantially decreased expression of aromatase compared with those in corresponding tissues from p53+/+ mice.
**Figure 1** p53 modulates lipid metabolism through transcriptionally regulating aromatase. (A) Shown are the representative image of p53<sup>−/−</sup> mice and their p53<sup>+/+</sup> littermates fed with the normal diet or HFD for a total of 13 weeks. The body mass of p53<sup>+/+</sup> and p53<sup>−/−</sup> mice fed with the normal diet or HFD was calculated and shown in the bottom part. (B) Shown are the representative images of liver and fat from p53<sup>+/+</sup> and p53<sup>−/−</sup> mice fed with the normal diet or HFD diet as indicated. The weight of livers from the indicated group of mice was also shown. (C) Schematic representation of the mouse aromatase genomic locus. The putative p53-binding site is indicated. Exons are indicated as gray boxes. Ar, aromatase; Pu, purine; Py, pyrimidine. Lysates from the p53<sup>+/+</sup> and p53<sup>−/−</sup> mouse liver tissue were subjected to chromatin immunoprecipitation with anti-p53, anti-H3 or control IgG antibody. (D) p53<sup>+/+</sup> and p53<sup>−/−</sup> MEF cells with or without aromatase stable knockdown by shRNA were seeded on coverslips and stained with ORO. The percentage of ORO-positive cells was calculated and shown in Supplementary Figure S4C. Aromatase knockdown efficiency was also confirmed by western blotting analysis (Supplementary Figure S4C). (E) p53<sup>+/+</sup> and p53<sup>−/−</sup> mice were crossed with aromatase transgenic (ArTG) to generate the p53<sup>+/+</sup> and ArTG and p53<sup>−/−</sup> and ArTG mice. The frozen sections of liver tissue from the indicated mice were stained with ORO. The percentage of the ORO-stained area was quantified by the NIS-Elements BR3.1 software (Nikon, Japan) and shown in Supplementary Figure S4D. The transgenic mice carrying the correct genes of interest was also confirmed by PCR analysis of mouse tail DNAs (Supplementary Figure S4D). (F) Frozen sections of liver tissue from p53<sup>+/+</sup> or p53<sup>−/−</sup> mice with vehicle oil or E<sub>2</sub> treatment were stained with ORO. The percentage of the ORO-stained area was quantified by the NIS-Elements BR3.1 software and shown in Supplementary Figure S4E. (G) A proposed model for the role of p53 in lipid metabolism. Aromatase, the key enzyme that converts testosterone to estrogen, is shown to be transcriptionally regulated by p53. In the absence of p53, the expression of aromatase is downregulated, thus leading to the inhibition of aromatase enzyme activity. This, in turn, results in the elevated level of serum testosterone and the increased ratio of T/E2, finally promoting lipid accumulation.
expression levels of aromatase were also decreased in p53−/− mice fed with the normal diet or HFD. Intriguingly, we found that the binding of p53 to the aromatase promoter was greatly inhibited by HFD treatment (Supplementary Figure S3D, lane 3 vs. 9). Correlating with this, mRNA expression levels of aromatase were also decreased in p53+/+ mouse liver upon HFD treatment (Supplementary Figure S3E, lane 1 vs. 3), while p53 levels were left unchanged in HFD-treated p53+/+ mouse liver (Supplementary Figure S3E, lane 1 vs. 3). In contrast, HFD treatment failed to show any effect on aromatase mRNA expression in p53−/− mouse liver (Supplementary Figure S3E, lane 2 vs. 4). These results indicate that long-term HFD treatment inhibits aromatase expression by decreasing binding of p53 to the aromatase promoter, and suggest the physiologically important function of the p53/ aromatase axis in regulating lipid metabolism.

We finally examined whether aromatase is essential for p53 in regulating lipid metabolism. In aromatase wild-type MEF cells, the lack of p53 resulted in an expected dramatic increase in the lipid content. However, when aromatase was knocked down, the promoting effect of p53 knockout on lipid accumulation was minimized (Figure 1D and Supplementary Figure S4C). Also, the ectopic expression of aromatase almost completely reversed the increase in the lipid content of p53 knockdown or p53 knockout MEF cells (Supplementary Figure S4A and B). To further determine the physiological significance of p53-mediated aromatase regulation in vivo, we generated aromatase transgenic mice, and crossed them with p53+/+ and p53−/− mice. As shown in Figure 1E, the liver from p53−/− mice exhibited a great increase in lipid droplets compared with that from p53+/+ mice, which was, however, almost completely reversed by transgenic expression of aromatase (Figure 1E and Supplementary Figure S4D). Previous study showed that hepatic lipid accumulation in aromatase knockout (ArKO) mice liver can be rescued by E2 replacement (Hewitt et al., 2004). Similarly, treatment of E2 resulted in the significantly reduced hepatic lipid droplets in the p53−/− mouse liver, but not in the p53+/+ mouse liver (Figure 1F and Supplementary Figure S4E). Taken together, these results demonstrate that aromatase is important for the function of p53 in the regulation of lipid metabolism.

Recent studies indicate that p53 is able to transcriptionally regulate several lipid metabolism-related genes (Goldstein et al., 2012) and suggest a possible link between p53 and obesity (Yahagi et al., 2003) and fatty liver disease (Yahagi et al., 2004; Derdak et al., 2011). However, the precise role of p53 in modulating lipid metabolism remains uncertain. Our study provides clear evidences that p53 regulates lipid metabolism through transcriptionally regulating aromatase. Similar to the phenotype of ArKO male mice, p53−/− mice exhibit the elevated T/E2 ratio and show the enhanced lipid accumulation in livers, especially under the HFD treatment. In this study, only male mice were used. The reasons were as follows. Firstly, p53−/− female mice were very hard to get due to the significant numbers of embryonic death of p53−/− female mice. Secondly, the phenotype of lipid accumulation in the ArKO mouse liver is specific to male mice. Therefore, whether p53 is also involved in the regulation of lipid metabolism in female mouse liver remains to be further determined. In our recent report, p53-mediated inhibition of lipid accumulation is partially due to its potent inhibitory activity against G6PD, a key regulatory point of PPP (Jiang et al., 2011). Therefore, p53 regulates lipid metabolism at least through two different mechanisms: one is G6PD dependent, and the other is dependent on aromatase. However it remains to be further investigated whether cross-talk exists between these two mechanisms. Since p53 has been shown to inhibit G6PD activity and NADPH production and high levels of NADPH have been shown to inactivate aromatase, it would be interesting to know whether p53 also regulates aromatase activity through G6PD- and NADPH-dependent mechanism. In addition, p53 has been recently revealed to enhance fatty acid oxidation and modulate lipid transport by inducing the expression of multiple genes (Goldstein and Rotter, 2012). These findings together with ours suggest that p53 could regulate various aspects of lipid metabolism via regulating different target genes expression, and implicate the complexity of p53 function in regulating lipid metabolism.

Here we propose a model for p53 in regulating lipid metabolism (Figure 1G). In this model, p53 deficiency leads to downregulation of aromatase expression, thus inhibiting aromatase enzyme activity. This, in turn, results in the elevated levels of serum testosterone and the increased ratio of T/E2 (McInnes et al., 2012), thereafter promoting lipid accumulation. Given the importance of p53 in negatively regulating lipid accumulation, it is conceivable that p53 may be a potential therapeutic target for various lipid metabolic disorders, such as obesity, diabetes, and liver steatosis.

[Supplementary material is available at Journal of Molecular Cell Biology online.

We thank N. Liang (University of Science and Technology of China) and P. Jiang (University of Pennsylvania School of Medicine) for technical assistance, Andrew Y.H. Tam (University of British Columbia) for editing the manuscript. This work was supported by National Natural Science Foundation of China grants 31030046 (to M.W.), the Ministry of Science and Technology of China grants (2010CB912804 and 2011CB966302) and the Fundamental Research Funds for Central Universities (USTC, WK2060190018).]
References


