# The Regulation of AMPK β1, TSC2, and PTEN Expression by p53: Stress, Cell and Tissue Specificity, and the Role of These Gene Products in Modulating the IGF-1-AKT-mTOR Pathways

Zhaohui Feng,[1] Wenwei Hu,[1] Elisa de Stanchina,[2] Angelika K. Teresky,[1] Shengkan Jin,[1] Scott Lowe,[2] and Arnold J. Levine[1,3]

[1]Cancer Institute of New Jersey, University of Medicine and Dentistry of New Jersey, New Brunswick, New Jersey; [2]Cold Spring Harbor Laboratories, Cold Spring Harbor, New York; and [3]School of Natural Sciences, Institute for Advanced Study, Princeton, New Jersey

## Abstract

**The insulin-like growth factor 1 (IGF-1)-AKT-mTOR pathways sense the availability of nutrients and mitogens and respond by signaling for cell growth and division. The p53 pathway senses a variety of stress signals which will reduce the fidelity of cell growth and division, and responds by initiating cell cycle arrest, senescence, or apoptosis. This study explores four p53-regulated gene products, the β1 and β2 subunits of the AMPK, which are shown for the first time to be regulated by the p53 protein, TSC2, PTEN, and IGF-BP3, each of which negatively regulates the IGF-1-AKT-mTOR pathways after stress. These gene products are shown to be expressed under p53 control in a cell type and tissue-specific fashion with the TSC2 and PTEN proteins being coordinately regulated in those tissues that use insulin-dependent energy metabolism (skeletal muscle, heart, white fat, liver, and kidney). In addition, these genes are regulated by p53 in a stress signal–specific fashion. The mTOR pathway also communicates with the p53 pathway. After glucose starvation of mouse embryo fibroblasts, AMPK phosphorylates the p53 protein but does not activate any of the p53 responses. Upon glucose starvation of E1A-transformed mouse embryo fibroblasts, a p53-mediated apoptosis ensues. Thus, there is a great deal of communication between the p53 pathway and the IGF-1-AKT and mTOR pathways.** [Cancer Res 2007;67(7):3043–53]

## Introduction

Cell growth and proliferation both require environmental or external signals showing the availability of adequate glucose, amino acids, as well as mitogens as signals for cell division. The levels of glucose and amino acids in the cell are monitored by the mTOR pathway. The LKB-1 and AMPK monitor the levels of glucose and ATP/AMP ratios in the cell. When glucose levels decrease below a threshold value, AMPK phosphorylates and activates the TSC2 protein (1–3). The TSC1/TSC2 protein complex is a GTPase which negatively regulates the RHEB G-protein (4), which in turn, positively regulates mTOR (4, 5). Thus, low glucose levels turn off mTOR activity and activates the process of autophagy (6, 7). Autophagic vesicles engulf cytoplasmic components and transport them to the lysosome wherein they are degraded to supply the cell with nutrients. The 4EBP1 protein and the S6 kinase are two major substrates of mTOR (8, 9). The 4EBP1 protein binds to and inactivates the translation initiation factor 4E, which is required for the translation of CAP-mRNAs (10). Phosphorylation of 4EBP1 by mTOR inactivates 4EBP1 and permits efficient translation. Phosphorylation and activation of S6 kinase by mTOR seems to enhance the translation of mRNAs involved in ribosomal biogenesis, mitochondrial biogenesis, as well as oxygen delivery and consumption (11, 12). Thus, mTOR mediates a switch between efficient translation of mRNAs that promotes energy production in the presence of glucose and catabolic degradation of existing organelles for the maintenance of the cell in the absence of glucose. This pathway is coregulated by the insulin-like growth factor 1 (IGF-1)-AKT pathway to ensure both a reasonable level of nutrients and a positive mitogen signal for cell growth and division. The IGF-I hormone binds to and activates the IGF receptor to recruit and activate the phosphoinositide-3-kinase to the cell membrane (13). The phosphoinositide-3-kinase produces PIP-3, which activates both the PDK-1 and mTOR-rictor kinases. That in turn phosphorylates and activates the AKT-1 kinase which moves into the cell nucleus. Phosphorylation of the FOXO transcription factors removes them from the nucleus, which changes their pattern of repression and transcription of genes (2, 13–15). In the presence of mitogens, heat shock chaperone proteins and antioxidant products favoring cell division cycles are produced, whereas in the absence of mitogens, inhibitors of cyclin-dependent kinases (p27) are produced (16). The IGF-I pathway coordinates with the mTOR pathway through the phosphorylation and inhibition of the TSC2 GTPase by AKT-1, which activates RHEB and mTOR (4, 17).

This coordinated commitment to cell division is monitored and regulated by several types of p53-mediated intrinsic and extrinsic stress signals, such as low levels of ribonucleoside triphosphate pools in the cell (18, 19), low levels of ribosome biogenesis (20), hypoxia, DNA damage, or even oncogene activation, each of which can introduce infidelity into the process of cell growth and division (21). In the presence of such stress signals, the p53 pathway shuts down both the IGF-1-AKT pathway and mTOR pathway (2, 22, 23). This article explores the set of p53-regulated genes: the β1 and β2 subunits of the AMPK, PTEN, TSC2, and the IGF-BP3, each of which is up-regulated in response to a stress signal in the cell and then negatively regulates the IGF-I-AKT-mTOR pathways in a p53-dependent fashion. In the extreme, these stress signals can result

**Note:** Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

**Requests for reprints:** Arnold J. Levine, School of Natural Sciences, Institute for Advanced Study, Einstein Drive, Princeton, NJ 08540. Phone: 609-734-8005; Fax: 609-924-7592; E-mail: alevine@ias.edu.



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in the coordinate activation of apoptosis and autophagy (22), and both processes have been shown to provide tumor suppressor activity (21, 24, 25). These four gene products that mediate the communication between p53 and the IGF-I-mTOR pathways are regulated by p53 in a tissue-specific and stress-specific fashion so that the tissues most responsive to insulin-dependent glucose utilization (skeletal muscle, heart, white fat, liver, and kidney) show this p53 regulation of the IGF-1-mTOR pathways. Glucose deprivation sensed by the AMPK initiates very different p53 responses in normal mouse embryo fibroblasts (MEF) or in oncogene-transformed fibroblasts, which communicate a stress signal to p53. In the absence of glucose, the transformed fibroblasts die of p53-mediated apoptosis, whereas the normal fibroblasts cease cell growth and division but do not die.

## Materials and Methods

**Cell lines and treatment of cells.** Human H460, H1299, WI38, MCF7, T47D, HepG2, Tera2 were obtained from American Type Culture Collection (Manassas, VA). HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells were generous gifts from Dr. B. Vogelstein at John Hopkins University. H1299-p53 and H1299-24 cells were generous gifts from Dr. C. Prives at Columbia University. E1A/ras-transformed MEF cells were established as previously described (26).

Cells were seeded in dishes 24 h prior to treatment and were 60% to 70% confluent at the time of treatment. Cells were subjected to different treatments and cultured for various times before harvest. For ionizing radiation, a 137Cs irradiator was used to deliver 10 to 25 Gy to cells. For UV treatment, culture medium was removed and cells were washed with PBS before irradiation by UV light (UV-C, 254 nm). ALLN (Calbiochem, La Jolla, CA), and actinomycin D were dissolved in ethanol, Adriamycin and taxol (Sigma, Saint Louis, MO) were dissolved in methanol, and PALA (NSC224131; National Cancer Institute, Bethesda, MD) was dissolved in water. Cells were treated with various concentrations of these chemicals for various times before being collected for Western blot and real-time PCR. For glucose starvation, normal MEF and E1A/ras-transformed MEF cells were cultured with complete medium for 24 h before being switched into medium without glucose. The apoptotic cells were stained with Annexin V using a Nexin kit, and analyzed with a flow cytometer (Guava Technologies, Inc., Hayward, CA) according to the instructions of the manufacturer.

**Mice and gamma-irradiation.** Four- to six-week-old C57BL/6 p53 knockout mice (27), and age- and sex-matched C57BL/6 wild-type controls (The Jackson Laboratory, Bar Harbor, ME) were subjected to 5 Gy of total body irradiation with a 137Cs gamma source. Mice were sacrificed at different time points after irradiation and different tissues were harvested for further experiments. At lease three mice were used for each group.

**Chromatin immunoprecipitation assay.** Chromatin immunoprecipitation (ChIP) assays were done using Upstate ChIP Assay Kit (Lake Placid, NY) according to the instructions of the manufacturer. The primer sets were designed to encompass the potential p53 binding elements in the human *AMPK β1* and *TSC2* gene. The sequences for the AMPK β1 gene are as follows: for promoter, 5′-TTCCCAATTAATTCACAAAACAAG-3′ and 5′-AGAATGGCAA AATGAATGGAATAC-3′; for exon 1, 5′-GGGTGGTGAAGCGGTTGGGAAAGT-3′ and 5′-GCGGAAGGAGTCGGAGCAC-3′; for intron 1, 5′-GATTTGGGTGGGGACGCAGAG-3′ and 5′-GAAGGGGCTACTACGAATCCAAGACTA-3′.

The sequences for the *TSC2* gene are as follows: for promoter, 5′-AGAGGCCCTGTCCGTGTCC-3′ and 5′-TCGCCCCAAGCAAGA AAA-3′; for intron 2a, 5′-CACGCCTGGCTAAGTTTTGTATTT-3′ and 5′-GGGTTTGGGCTGGCTCTCAC-3′; for intron 2b, 5′-CATGGCGAAACCCCGTCTGT-3′ and 5′-AGCGAGTGAGCATGCGTTTCTATC-3′; for intron 11, 5′-GGCCGGGTGC TGGGTGAAGTG-3′ and 5′-GCCTGGGCCCTAAGCTGAGTGTT-3′; for p53 binding element in *Mdm2* promoter, 5′-GGTTGACTCAGCTTTTCCTCTTG-3′ and 5′-GGAAAATGCATGGTTTAAATAGCC-3′.

**Construction of reporter plasmids and luciferase activity assay.** The TOPO II vector (Invitrogen, Carlsbad, CA) was used to clone PCR fragments containing the putative p53 binding elements in human *AMPK β1, TSC2* gene by using PCR primers for ChIP assays. The sequence-confirmed clones were subcloned into pGL2 luciferase reporter plasmid (Promega, Madison, WI) at *Xho*I and *Hin*dIII sites. The pGL2 reporter plasmids containing one copy of each putative p53 binding element were transfected into p53 null HCT116 $p53^{-/-}$, Saos2 cells by using Lipofectamine 2000 (Invitrogen) along with 1 μg of pRC-wtp53 (wild-type human p53 expression plasmid) or pRC-273H (mutant human p53 expression plasmid containing a substitution at R273H) and 0.5 ng of pRL-SV40 plasmid expressing renilla luciferase as internal control to normalize transfection efficiency. The luciferase activity was measured 24 h after transfection. The reporter activity was calculated as luciferase activity of reporter plasmids in cells with wild-type p53 compared with that in cells with mutant p53.

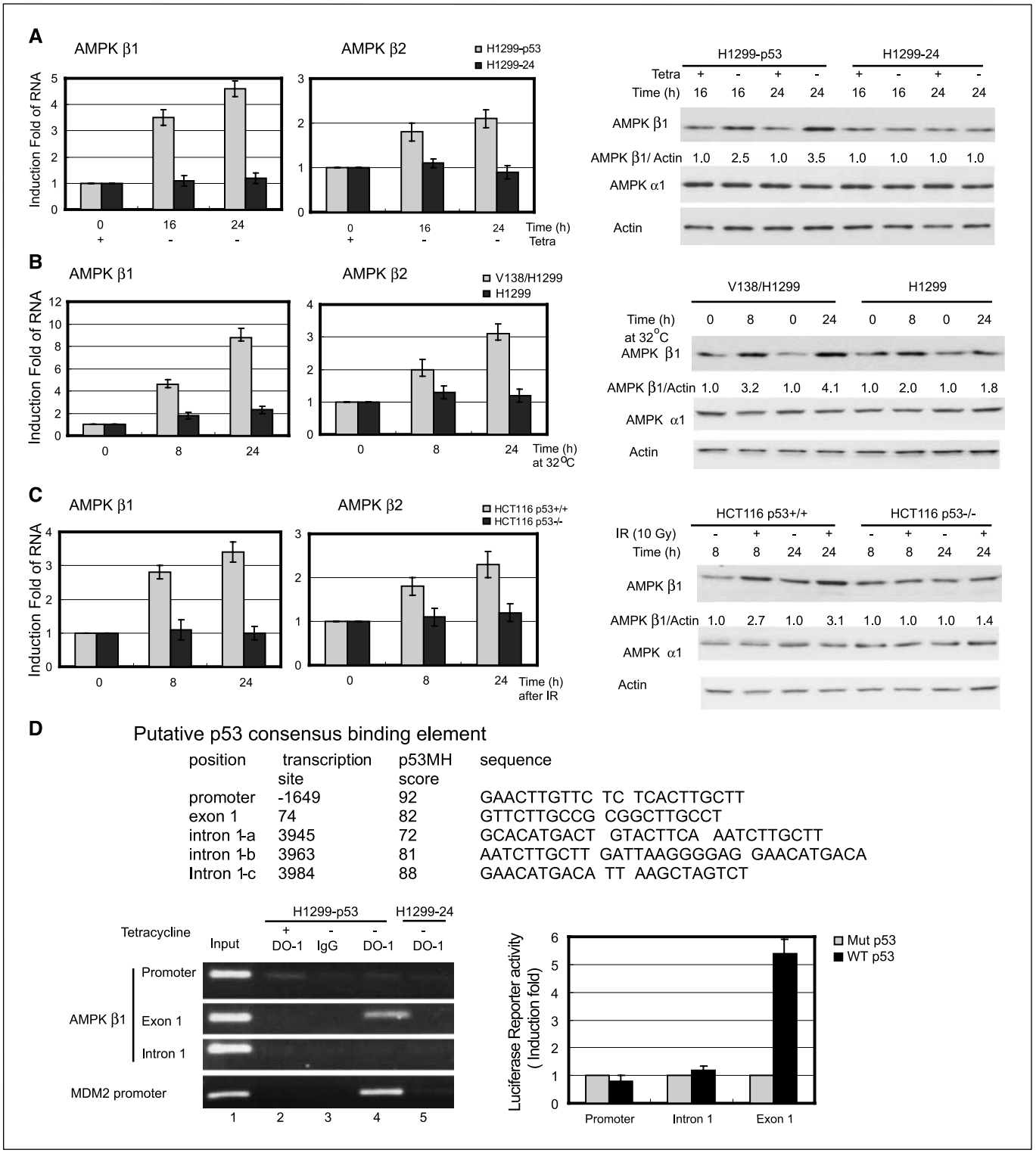
**Western blot analysis.** Standard Western blot analysis was used to analyze protein expression. Anti-human p53 monoclonal antibody DO-1 (sc-126), anti-TSC2 (sc-893), and anti-PTEN (sc-7974) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-human AMPK α (2532) and anti-AMPK β1 (4182) were purchased from Cell Signaling (Danvers, MA). Anti-β-actin (A5441) was purchased from Sigma. The protein levels were quantified by digitalization of the X-ray film and analyzed with Scion Image software (Scion Corporation, Frederick, MD).

**Quantitative real-time PCR.** Total RNA was prepared from cells or mouse tissues with the RNeasy kit (Qiagen, Valencia, CA) and treated with the DNase I to remove residual genomic DNA. The cDNA was prepared with random primers using TaqMan reverse transcription kit (Applied Biosystems, Foster City, CA). Real-time PCR was done in triplicate with TaqMan PCR mixture (Applied Biosystems) for 15 min at 95°C for initial denaturing, followed by 40 cycles of 95°C for 30 s and 60°C for 30 s in the 7000 ABI sequence detection system. All human and mouse primers were purchased from Applied Biosystems. The expression of genes was normalized to the housekeeping β-actin gene.

## Results

**Human *AMPK β1* and *TSC2* genes contain multiple putative p53 consensus binding elements.** The p53 protein is a transcription factor that specifically recognizes and binds to degenerate DNA consensus sequences loosely defined as PuPuPuC(A/T) (T/A)GPyPyPy $(N)_{0\text{-}14}$ PuPuPuC(A/T) (T/A)GPyPyPy, in which Pu stands for purine, Py stands for pyrimidine, and N stands for any nucleotide (28). Recently, Hoh et al. (29) developed a novel algorithm (p53MH) for genome-wide scanning for putative p53 consensus binding elements. Employing this program, multiple putative p53 consensus binding elements were found in human *AMPK β1* and *TSC2* genes as shown in Figs. 1*D* and 2*D*, suggesting that these two genes are possible p53-regulated genes.

***AMPK β1* is a p53-regulated gene.** As a heterotrimeric complex, AMPK consists of one catalytic α subunit and two regulatory β and γ subunits. Each subunit is encoded by multiple genes (α1, α2, β1, β2,γ1, γ2, γ3; ref. 30). The β subunits function as a scaffold for the binding of α and γ subunits. In addition, it has been shown that β subunits modulate AMPK activity and its cellular localization (31). To test if AMPK β1 is a p53-regulated gene, the ability of p53 to regulate the expression of *AMPK β1* was examined in various human cell lines. H1299-p53 cells were established by stably transfecting a p53 plasmid (pUHD15-1-neo) into p53 null human lung epithelial H1299. The plasmid contains a wild-type p53 cDNA under a tetracycline-regulated promoter, which expresses wild-type p53 protein in the absence of tetracycline although not in the presence of tetracycline (1 μg/mL;




**D**

Putative p53 consensus binding element

| position | transcription site | p53MH score | sequence |
|---|---|---|---|
| promoter | -1649 | 92 | GAACTTGTTC TC TCACTTGCTT |
| exon 1 | 74 | 82 | GTTCTTGCCG CGGCTTGCCT |
| intron 1-a | 3945 | 72 | GCACATGACT GTACTTCA AATCTTGCTT |
| intron 1-b | 3963 | 81 | AATCTTGCTT GATTAAGGGGAG GAACATGACA |
| Intron 1-c | 3984 | 88 | GAACATGACA TT AAGCTAGTCT |




**Figure 1.** p53 regulates the expression of AMPK β1 and AMPK β2 genes in human cells. *A* to *C,* p53 was activated in cultured human cells, the RNA and protein levels of AMPK β1 and AMPK β2 were measured by TaqMan quantitative real-time PCR and Western blot, respectively. The levels of AMPK β1 and AMPK β2 were normalized against the levels of β-actin. *A,* H1299-p53 cells and H1299-24 cells were cultured in the presence or absence of 1 μg/mL of tetracycline (*Tetra*) for 24 or 36 h. *B,* V138/H1299 and H1299 cells were shifted from 37°C to 32°C for the indicated number of hours before collecting. *C,* human colon epithelial HCT116 $p53^{+/+}$ and $p53^{-/-}$ cells were gamma-irradiated (10 Gy), and cultured for the indicated number of hours before collecting. *D,* the p53 protein binds to and transactivates the p53 consensus binding sequences in the human AMPK β1 gene. In the ChIP assay, H1299-p53 and H1299-24 cells were cultured in the presence or absence of tetracycline (1 μg/mL) for 24 h followed by ChIP assay with DO-1 anti-p53 antibody. DNA (1/20) of ChIP was used for PCR as Input DNA (*lane 1*). The p53 binding element in MDM2 promoter was used as a positive control. In the luciferase reporter assay, DNA fragments containing the putative p53 binding elements were inserted into pGL2 luciferase reporter plasmid. The constructed plasmids were transfected into HCT116 $p53^{-/-}$ cells along with a wild-type (*WT p53*) or 273H mutant (*Mut p53*) p53 expression plasmid and an internal standard pRL-SV40 plasmid. Luciferase activity was measured 24 h after transfection. The reporter activity was calculated as luciferase activity of reporter plasmids in cells cotransfected with wild-type p53 compared with that in cells cotransfected with mutant p53.

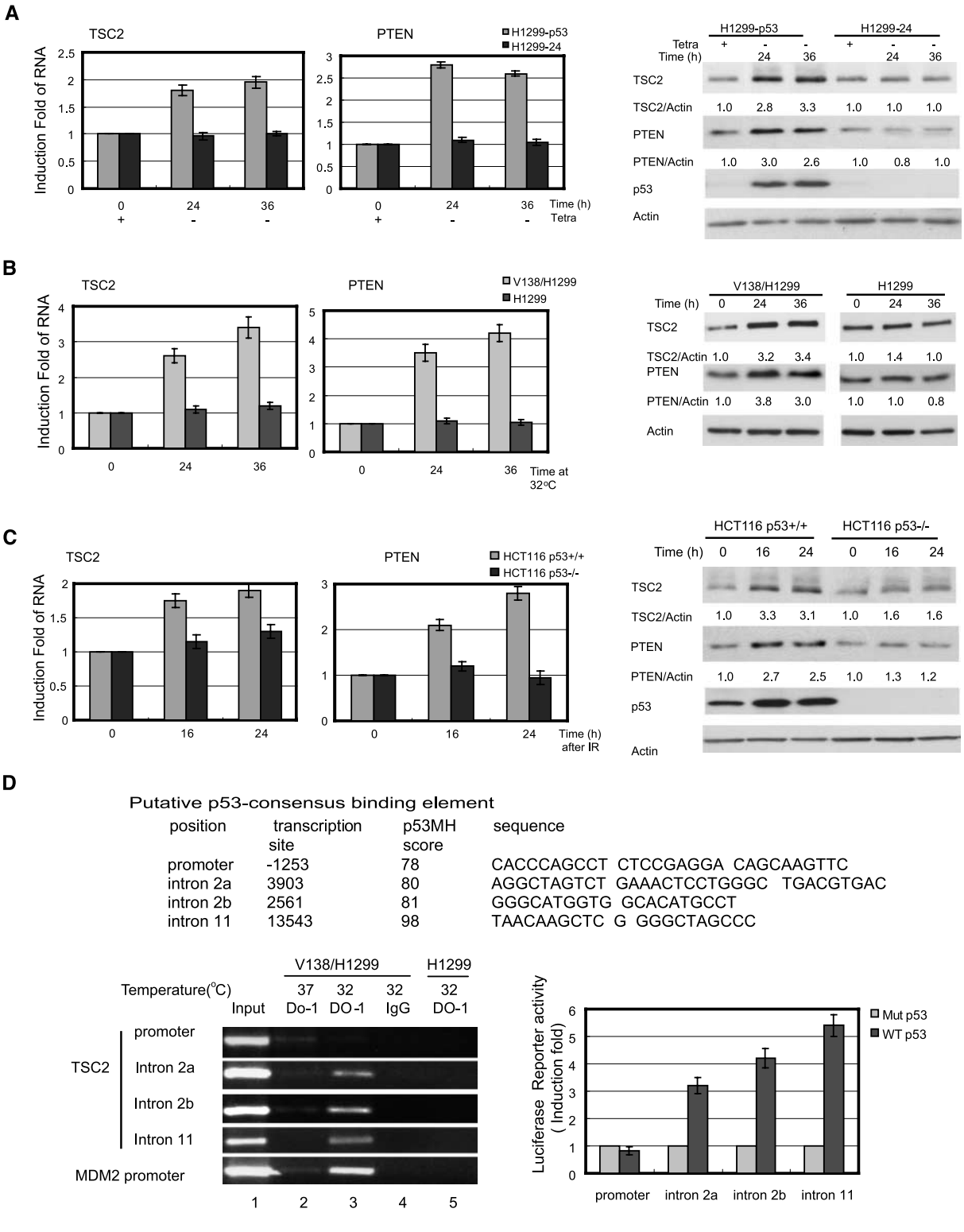



**Figure 2.** p53 regulates the expression of TSC2 and PTEN genes in human cells. *A* to *C,* p53 was activated in cultured human cells (Fig. 1), and the RNA and protein levels of TSC2 and PTEN were measured by TaqMan quantitative real-time PCR and Western blot, respectively. The levels of TSC2 and PTEN were normalized against the levels of actin. *A,* H1299-p53 and H1299-24 cells; *B,* V138/H1299 and H1299 cells; *C,* HCT116 $p53^{+/+}$ and $p53^{-/-}$ cells; *D,* the p53 protein binds to and transactivates the p53-consensus binding elements in the human TSC2 gene. In the ChIP assay, V138/H1299 and H1299 cells were cultured at 32°C for 24 h followed by ChIP assay with DO-1 anti-p53 antibody or IgG (*lanes 2–5*). DNA (1/20) of ChIP was used for PCR as Input DNA (*lane 1*). In the luciferase reporter assay, the constructed pGL2 luciferase reporter plasmids containing the putative p53 binding elements in the TSC2 gene were transfected into HCT116 $p53^{-/-}$ cells along with a WT p53 or 273H Mut p53 expression plasmid and an internal standard pRL-SV40 plasmid. Luciferase activity was measured 24 h after transfection. The reporter activity was calculated as in Fig. 1D.

ref. 32). As a control, the H1299-24 cell line was established by stably transfecting H1299 cells with pUHD15-1-neo plasmid, which does not contain p53 sequences. The RNA levels of AMPK β1 were measured by TaqMan quantitative real-time PCR before and after tetracycline withdrawal. As shown in Fig. 1*A*, the RNA levels of *AMPK β1* were significantly increased by 3.5- to 4.6-fold after p53 activation in H1299-p53 cells. A homologue of *AMPK β1* gene (66% homologous of nucleotides), the *AMPK β2* gene, was also transcriptionally induced by p53 activation, but increased much less (~2-fold). In contrast, no induction was observed for α1, α2, γ1, or γ2 subunits of AMPK in H1299-p53 cells after tetracycline withdrawal (Supplementary Table S1), and no induction of *AMPK β1* or *AMPK β2* RNA was observed in H1299-24 cells expressing no p53 protein (Fig. 1*A*). The induction of AMPK β1 mRNA expression by p53 in H1299-p53 cells was also confirmed at the protein level by Western blots as shown in Fig. 1*A*, whereas no induction of AMPK β1 protein was observed in the control H1299-24 cells.

The regulation of *AMPK β1* by p53 was further investigated in H1299/V138 cells. H1299/V138 cells were established by stably transfecting a temperature-sensitive mutant form of p53 (alanine 138 to valine) into p53 null H1299 cells (33). As shown in Fig. 1*B*, when p53 was activated after H1299/V138 cells were shifted from 37°C to 32°C, the expression of *AMPK β1* and *AMPK β2* was significantly induced at the RNA level (4.6- to 8.8-fold for *AMPK β1*, and 2.2- to 3.0-fold for *AMPK β2*), whereas no induction was observed for α1, α2, γ1, or γ2 subunits (Supplementary Table S1). It is worth noting that cold shock itself could also induce AMPK β1 mRNA expression to a low level in a p53-independent manner as observed in p53-null H1299 cells. However, compared with the induction of *AMPK β1* in H1299/V138 cells, the p53-dependent induction was much more significant than the p53-independent induction (4.6- versus 1.8-fold at 6 h, and 8.8- versus 2.3-fold at 24 h), which was also observed at protein level as shown in Fig. 1*B*. This p53-independent induction of *AMPK β1* expression was previously reported (34).

HCT116 $p53^{+/+}$ is a human colon carcinoma cell line containing a wild-type p53 gene, whereas HCT116 $p53^{-/-}$ is a p53 knockout cell line derived from HCT116 $p53^{+/+}$ by homologous recombination. As shown in Fig. 1*C*, the expression of *AMPK β1* was significantly increased at both RNA and protein levels after p53 activation by gamma-irradiation (10–25 Gy). As a control, no induction of RNA or protein levels of AMPK β1 was observed in HCT116 $p53^{-/-}$ cells after irradiation. These data showed that *AMPK β1* expression was transcriptionally up-regulated by p53, suggesting that AMPK β1 is a p53-regulated gene.

To determine if p53 can bind to these putative p53 binding elements in human *AMPK β1* gene *in vivo*, a ChIP assay was done. As shown in Fig. 1*D*, H1299-p53 cells were cultured in the absence of tetracycline for 24 h to induce p53 expression and the ChIP assay was done with a monoclonal anti-p53 antibody, DO-1. Immunoprecipitation of the chromatin fragment corresponding to the binding element in exon 1 (~200 bases in front of the first codon) was observed in the H1299-p53 cells cultured without tetracycline, but not for the binding elements in the promoter or in intron 1 (the three binding elements in intron 1 are overlapped and only one pair of PCR primers were employed to amplify the DNA fragment). In contrast, these chromatin fragments could not be coimmunoprecipitated with the DO-1 antibody in either H1299-p53 cells cultured in the presence of tetracycline or H1299-24 cells cultured in the absence of tetracycline, which did not express p53 protein. These results clearly showed that p53 interacted with a putative p53 consensus binding element in exon 1 of the human AMPK β1 gene *in vivo*.

To test if these p53 consensus-binding elements could confer p53-dependent transcriptional activity, the DNA fragments containing one copy of the above putative binding elements were inserted into a pGL2 luciferase reporter plasmid. The HCT116 $p53^{-/-}$ cells were cotransfected with these constructed reporter plasmids and a plasmid expressing either wild-type (pRC p53) or mutant p53 (pRC 273H). pRL-SV40-TK plasmids were cotransfected as an internal standard to normalize transfection efficiency. The results in Fig. 1*D* showed that compared with mutant p53 protein, wild-type p53 enhanced luciferase expression by ~5-fold in the reporter plasmid containing the putative p53 binding element in exon 1, although not in reporter plasmids containing other putative p53 binding elements. These results were consistent with the results of the ChIP assay. Taken together, these data indicate that AMPK β1 is a target of p53, which can be directly regulated by p53 through sequence-specific DNA binding and transcriptional activation.

**TSC2 is a p53-regulated gene.** Previously, it has been shown that *TSC2* expression could be induced by p53 activation in temperature-sensitive V138/H1299 cells, which suggested that *TSC2* could be a p53 target gene (22). To extend these results, the regulation of TSC2 by p53 was further investigated in the H1299-p53 after tetracycline withdrawal, and in irradiation-treated HCT116 $p53^{+/+}$ cells. As shown in Fig. 2*A* to *C*, moderate but consistent induction of TSC2 RNA levels (~1.8- to 3-fold) were observed in these cells. In addition, the induction of protein was also observed. In contrast, no induction of TSC2 at either RNA or protein levels was observed in their control cells: H1299-24 cells without tetracycline or H1299 cells at 32°C, which expressed no wild-type p53. In irradiation-treated HCT116 $p53^{-/-}$ cells, marginal induction of TSC2 was also observed at both RNA and protein levels (Fig. 2*C*). However, the induction fold was much less compared with that in irradiation-treated HCT116 $p53^{+/+}$ cells, suggesting that the p53-dependent induction accounted for the major expression induction of TSC2 after irradiation treatment.

To determine if p53 can bind to the putative p53 consensus binding elements in human *TSC2* gene *in vivo*, ChIP assays were done in V138/H1299 cells, which showed the highest induction of *TSC2* by p53 activation. Because *TSC2* contains 42 exons, the binding element in intron 11 was also tested for its possible binding ability to p53. V138/H1299 cells were shifted to 32°C for 24 h to activate p53 before ChIP assays were done with anti-p53 antibody. As shown in Fig. 2*D*, immunoprecipitation of chromatin fragments corresponding to both elements in intron 2 and the one in intron 11 were observed but was not detected in the promoter. DNA fragments containing one copy of the above *TSC2* binding elements were then inserted into the pGL2 luciferase reporter plasmid, and transfected into HCT116 $p53^{-/-}$ cells along with the wild-type p53 expression plasmid or the mutant p53 273H plasmid. The results in Fig. 2*D* show that compared with mutant p53 protein, wild-type p53 enhanced luciferase expression by ~3-, 4.5-, 5.5-fold in the reporter plasmids containing *TSC2* intron 2a, intron 2b, and intron 11 elements, respectively, although not in the plasmid containing the element from the promoter. Similar results were also observed in p53 null human osteosarcoma Saos2 cells (data not shown). These data confirm the previous suggestion that the TSC2 gene is truly a p53-regulated gene (22).

**PTEN is a p53-regulated gene.** Previously, it was reported that *PTEN* could be regulated by p53 (35), but several additional studies found inconsistent results (36, 37). To see whether *PTEN* is really a p53-regulated gene, the expression levels of PTEN were measured after p53 activation in the abovementioned cells. As shown in Fig. 2*A* to *C*, PTEN was clearly up-regulated by p53 at both the RNA and protein levels after p53 activation in H1299-p53 cells after tetracycline withdrawal, in V138/H1299 cells at 32°C, and in irradiation-treated HCT116 $p53^{+/+}$ cells. Its induction was p53-dependent because an increase in RNA or protein was never observed in the control cells expressing no wild-type p53. These results clearly showed that *PTEN* is a p53-regulated gene.

To explore the inconsistencies in the literature concerning the p53 regulation of the PTEN gene, we tested whether or not this gene was p53-regulated in a cell type–specific fashion. The RNA levels of the *PTEN* gene as well as the *TSC2* gene were monitored by real-time PCR in a number of different cell lines with different p53 status after exposure to irradiation. The results presented in Table 1 show that the induction of both genes seemed to be cell type–specific. Clear induction of both genes at transcriptional levels was seen in p53 wild-type HCT116 $p53^{+/+}$, H460, WI38, U2OS, and HeLa cells, whereas only marginal or no induction was seen in p53 wild-type Tera 1, MCF7, A172, A293, HepG2, and MEF $p53^{+/+}$ cells, or in p53 null or mutant cells including HCT116 $p53^{-/-}$, H1299, T47 D, and MEF $p53^{-/-}$ cells. These results may help to explain the previous observations that the regulation of PTEN by p53 was only observed under certain circumstances. More interestingly, *TSC2* and *PTEN* genes seemed to be coordinately regulated by p53: cell lines that transcriptionally activated the *TSC2* gene after irradiation also induced *PTEN* transcription, whereas cell lines that failed to regulate *TSC2* in a p53-dependent fashion also failed to regulate *PTEN*.

**Table 1.** Gene induction in various cell lines 24 h after gamma-irradiation

| Cell line | p53 status | Tissue source | TSC2 | PTEN |
|---|---|---|---|---|
| HCT116 $p53^{+/+}$ | WT | Colon | 1.9 ± 0.1 | 2.8 ± 0.2 |
| H460 | WT | Lung | 2.3 ± 0.2 | 2.4 ± 0.1 |
| WI38 | WT | Lung | 1.8 ± 0.1 | 1.9 ± 0.1 |
| U2OS | WT | Bone | 1.7 ± 0.1 | 1.8 ± 0.1 |
| HeLa | WT-HPV | Cervix | 1.6 ± 0.1 | 2.1 ± 0.2 |
| HepG2 | WT | Liver | 1.3 ± 0.1 | 1.5 ± 0.2 |
| MCF7 | WT | Breast | 1.3 ± 0.1 | 1.3 ± 0.1 |
| A172 | WT | Brain | 1.0 ± 0.1 | 1.5 ± 0.2 |
| Tera1 | WT | Testes | 0.9 ± 0.1 | 1.0 ± 0.1 |
| A293 | WT | Kidney | 1.0 ± 0.1 | 0.9 ± 0.1 |
| HCT116 $p53^{-/-}$ | Null | Colon | 1.3 ± 0.1 | 0.9 ± 0.2 |
| H1299 | Null | Lung | 1.4 ± 0.2 | 1.1 ± 0.1 |
| T47D | Mutant | Breast | 1.2 ± 0.1 | 1.1 ± 0.2 |
| MEF $p53^{+/+}$ | WT | MEF | 1.3 ± 0.1 | 1.1 ± 0.1 |
| MEF $p53^{-/-}$ | Null | MEF | 1.4 ± 0.1 | 1.2 ± 0.1 |

NOTE: All cell lines listed are human cells except MEF. Cells were treated with 10 Gy of gamma-irradiation and harvested at different time points (5, 10, and 24 h) postirradiation. The RNA levels were determined by TaqMan quantitative real-time PCR. All expression levels were normalized to actin. Gene induction was calculated as gene expression levels in irradiated cells compared with untreated control cells. Because the highest expression of both genes was detected at 24 h postirradiation, only results at 24 h were presented.

**Stress-specific induction of AMPK β1, TSC2, and PTEN expression by p53.** The p53 protein plays a central role in integrating various stress signals and can be activated by various stresses (21, 38). In addition to DNA-damaging agents, p53 can be activated by hypoxia, heat shock, microtubule-active drugs, thymidine dinucleotides, or perturbations of nucleotide pools (19). To study the response of *AMPK β1* and *AMPK β2, TSC2,* and *PTEN* to p53 activation by different genotoxic and nongenotoxic stress signals, various kinds of stress were employed to treat HCT116 $p53^{+/+}$ and $p53^{-/-}$ cells to activate p53. The RNA levels of these genes were then detected in the cells before and after the treatment. MDM2 expression levels were also detected as a positive control. The stresses included: (*a*) UV, which causes DNA base damage; (*b*) Adriamycin, which induces DNA double-stranded breaks; (*c*) low levels of actinomycin D, an inhibitor of RNA synthesis that at low levels blocks rRNA synthesis; (*d*) ALLN, an inhibitor of ubiquitin-mediated degradation by the 26 S proteasome; (*e*) Taxol, which disrupts microtubules; (*f*) PALA, which causes depletion of ribonucleotides without detectable DNA damage. The results presented in Table 2 show that the induction of *AMPK β1* and *AMPK β2, TSC2,* and *PTEN* expression by p53 activation was stress-specific. The AMPK β1 expression was induced by UV in a p53-dependent manner, by actinomycin D in both p53-dependent and -independent manners, and by Adriamycin in mainly a p53-independent manner. The induction patterns of *AMPK β2* expression by various stresses were very similar as *β1* gene except for the lower induction levels. The expression of *TSC2* and *PTEN* seemed to be coordinately regulated by each stress signal employed except for PALA. Both genes were transcriptionally induced by Adriamycin and actinomycin D, whereas not by UV, ALLN, or Taxol. A marginal p53-independent induction of *TSC2* was observed in HCT116 $p53^{-/-}$ cells by some stresses, such as actinomycin D and Adriamycin, however, the induction levels were much lower when compared with that in $p53^{+/+}$ cells. The coordinated regulation of TSC2 and PTEN by p53 upon various stresses was also observed at protein level (Supplementary Fig. S1).

**Tissue-specific induction of *TSC2, PTEN,* and *IGF-BP3* expression by p53 in mice.** Previous studies have shown that different types of mouse cells and tissues respond differently after exposure to irradiation. Some tissues induce high levels of p53 and undergo apoptosis, some tissues induce high levels of p53 and undergo $G_1$ arrest, whereas some other tissues do not have increased p53 levels (39, 40). We next explored the tissue specificity in mice of the p53-regulated inhibition of the IGF-1-AKT-mTOR pathways.

*AMPK β1, TSC2, PTEN,* and *IGF-BP3,* four p53 target genes, are important negative regulators in the IGF-1-AKT-mTOR pathways. To map the patterns of their induction by p53 in mouse tissues, the RNA expression levels of these genes were analyzed in a wide variety of tissues from gamma-irradiated (5 Gy) p53 wild-type and $p53^{-/-}$ mice using quantitative real-time PCR. The induction patterns of another group of the p53 target genes that form negative feedback loops with p53 were also analyzed to compare whether these two subgroups of the p53 target genes had distinct induction patterns in mouse tissues. This group of genes included *Mdm2, cyclin $G_1$, Wip1* as well as *Pirh2 and Cop1,* two newly

**Table 2.** Gene induction in HCT116 $p53^{+/+}$ or $p53^{-/-}$ cells after various stresses

| Treatment | Cell | Dosage | TSC2 | PTEN | AMPK β1 | AMPK β2 | Mdm2 |
|---|---|---|---|---|---|---|---|
| Actinomycin D (nmol/L, 15 h) | $p53^{+/+}$ | 1 | 1.7 ± 0.2 | 1.9 ± 0.1 | 5.2 ± 0.3 | 3.0 ± 0.3 | 11.3 ± 0.7 |
| | $p53^{+/+}$ | 3 | 2.1 ± 0.2 | 2.3 ± 0.2 | 8.0 ± 0.5 | 4.5 ± 0.3 | 9.6 ± 0.8 |
| | $p53^{-/-}$ | 1 | 0.8 ± 0.1 | 0.9 ± 0.1 | 2.1 ± 0.2 | 1.8 ± 0.2 | 1.6 ± 0.2 |
| | $p53^{-/-}$ | 3 | 1.2 ± 0.1 | 1.3 ± 0.1 | 3.2 ± 0.2 | 2.5 ± 0.3 | 2.1 ± 0.2 |
| PALA (μmol/L, 24 h) | $p53^{+/+}$ | 50 | 1.5 ± 0.1 | 0.9 ± 0.1 | NA | NA | 4.3 ± 0.4 |
| | $p53^{+/+}$ | 100 | 1.8 ± 0.2 | 1.2 ± 0.1 | NA | NA | 6.9 ± 0.6 |
| | $p53^{-/-}$ | 50 | 1.1 ± 0.1 | 0.8 ± 0.1 | NA | NA | 1.1 ± 0.1 |
| | $p53^{-/-}$ | 100 | 1.0 ± 0.1 | 0.9 ± 0.1 | NA | NA | 1.4 ± 0.1 |
| Adriamycin (ng/mL, 24 h) | $p53^{+/+}$ | 50 | 1.6 ± 0.2 | 1.8 ± 0.2 | 3.1 ± 0.3 | 1.9 ± 0.2 | 6.3 ± 1.0 |
| | $p53^{+/+}$ | 150 | 1.7 ± 0.1 | 1.5 ± 0.1 | 3.4 ± 0.2 | 2.1 ± 0.2 | 4.8 ± 0.5 |
| | $p53^{-/-}$ | 50 | 1.2 ± 0.2 | 0.9 ± 0.1 | 2.3 ± 0.2 | 1.4 ± 0.1 | 2.1 ± 0.5 |
| | $p53^{-/-}$ | 150 | 1.3 ± 0.2 | 0.9 ± 0.2 | 2.5 ± 0.2 | 1.7 ± 0.2 | 1.3 ± 0.1 |
| Taxol (nmol/L, 24 h) | $p53^{+/+}$ | 20 | 1.0 ± 0.1 | 1.1 ± 0.1 | 0.9 ± 0.1 | 1.0 ± 0.1 | 3.2 ± 0.2 |
| | $p53^{+/+}$ | 40 | 0.9 ± 0.1 | 1.0 ± 0.1 | 1.1 ± 0.2 | 0.9 ± 0.2 | 4.9 ± 0.3 |
| ALLN (μmol/L, 24 h) | $p53^{+/+}$ | 10 | 0.8 ± 0.1 | 0.6 ± 0.1 | 1.4 ± 0.1 | 1.2 ± 0.1 | 3.2 ± 0.2 |
| | $p53^{+/+}$ | 30 | 0.9 ± 0.1 | 0.5 ± 0.1 | 1.6 ± 0.1 | 1.1 ± 0.2 | 9.2 ± 0.6 |
| UV ($J/m^2$, 24 h) | $p53^{+/+}$ | 10 | 1.0 ± 0.1 | 0.8 ± 0.1 | 3.8 ± 0.2 | 2.3 ± 0.3 | 2.1 ± 0.2 |
| | $p53^{+/+}$ | 20 | 1.0 ± 0.2 | 0.8 ± 0.1 | 4.2 ± 0.3 | 2.5 ± 0.3 | 2.9 ± 0.2 |
| | $p53^{-/-}$ | 10 | 1.1 ± 0.1 | 1.0 ± 0.1 | 1.3 ± 0.2 | 1.3 ± 0.2 | 1.0 ± 0.1 |
| | $P53^{-/-}$ | 20 | 0.9 ± 0.1 | 1.1 ± 0.1 | 1.4 ± 0.2 | 1.2 ± 0.2 | 1.1 ± 0.1 |

NOTE: Cells were treated with different stresses for different times before harvest. The RNA levels were determined by TaqMan quantitative real-time PCR. All expression levels were normalized to actin. Gene induction was calculated as gene expression levels in treated cells compared with control cells treated with dissolvents.
Abbreviation: NA, not analyzed.

identified p53-targeted genes that have p53-ubiqutin ligase activity (41, 42). The induction of these genes after irradiation treatment was measured in different tissues and listed in Table 3A and B. These data show that RNA levels of *TSC2, PTEN,* and *IGF-BP3* were up-regulated by irradiation in a p53-specific fashion because increased RNA levels were observed only in $p53^{+/+}$ tissues (Table 3A), whereas no induction or much less induction was observed in $p53^{-/-}$ tissues (Supplementary Table S2). The induction of *TSC2* and *PTEN* genes showed a distinct tissue-specific pattern. Unlike p21 and MDM2 genes which were induced significantly in almost all $p53^{+/+}$ tissues with the highest induction in radiosensitive tissues including spleen and thymus, *TSC2* and *PTEN* were only significantly induced in tissues in which insulin-dependent glucose utilization occurs in energy metabolism such as white fat, skeletal muscle, heart, liver, and kidney, in which the IGF-1-AKT-mTOR pathways act in response to exogenous glucose levels (30). In contrast, no mRNA induction of the *TSC2* and *PTEN* genes was observed in spleen or thymus. More interestingly, *TSC2* and *PTEN* were coordinately regulated by p53 activation in each of these mouse tissues, consistent with the observations from different cell lines or with different stresses. Although IGF-BP3 is also a negative regulator in IGF-1-AKT-mTOR pathways, its induction pattern was very different from that of *TSC2* and *PTEN* genes, with the highest levels of mRNA in spleen and thymus. This might be due to the special property of IGF-BP3 as a secreted protein, which is released into the bloodstream from active lymphocytes in the spleen and the thymus, and binds to IGF-1 in the serum or fluids to regulate the IGF-1-AKT-mTOR pathways (43). It is worth noting that no clear induction of *AMPK β1* expression was observed in the $p53^{+/+}$ tissues analyzed (data not shown). Searching the mouse *AMPK β1* gene for the putative p53 consensus-binding elements revealed that in exon 1 (~200 bases in front of the first codon) there are homologous nucleotide sequences as in the p53-binding element in exon 1 of the human gene. However, there are differences at several bases which are essential for the p53 binding affinity [degenerate DNA consensus sequences: PuPuPu**C(A/T)(T/A)G**PyPyPy $(N)_{0\text{-}14}$ PuPuPu**C(A/T)(T/A)G**PyPyPy; the essential bases are highlighted]; in human: GTTCTTGCCG CGGCTTGCCT; and in mouse: GTTCCAGTCG CGG_TCGCCC. This may be the reason why the mouse *AMPK β1* gene was not a regulated target for p53. By contrast, the *MDM2* and *cyclin $G_1$* genes seemed to be highly induced in almost all tissues, and Wip1 was moderately induced in the majority of tissues, suggesting the importance of a p53-negative feedback loop in regulating the p53 activity in all tissues after stress. The induction of *Pirh2* showed clear tissue specificity; moderate but clear induction of *Pirh2* expression was only observed in some tissues. Although, the overall induction levels were much less for *Pirh2* compared with that of *Mdm2,* this tissue-specific induction of *Pirh2* expression suggested that *Pirh2* might only play a role in these specific tissues unlike the more general MDM2. No clear induction of *Cop1* expression was observed in the entire array of the mouse tissues analyzed. These results showed the extraordinary tissue specificity of the response of different p53 target genes after stress.

**Glucose starvation induces p53-dependent apoptosis in E1A/Ras-transformed MEF cells.** In addition to the crosstalk from p53 to the IGF-1-AKT-mTOR pathways initiated by the p53 activation after a stress signal, it has also been shown that these

pathways can communicate in the other direction, by a change in nutrient levels (glucose starvation) or activation of AMPK by the activator, AICAR (22, 44). Depletion of glucose, the major direct energy source for mammalian cells, resulted in AMPK activation and in turn resulted in the rapid but transient phosphorylation of the p53 protein at $Ser^{15}$, the first step in p53 activation (22). In normal MEF cells ($p53^{+/+}$ and $p53^{-/-}$), this transient change, induced by glucose starvation, did not stabilize p53 nor did it result in a full p53 response of apoptosis. However, when MEF cells were transformed with activated oncogenes E1A, which bind to Rb protein and liberates E2F-1 to activate p19 ARF, glucose starvation resulted in full p53 stabilization response and triggered p53-dependent apoptosis and cell death (Fig. 3*A*). Thus, oncogene activation in a cell creates a communication between the p53 and the mTOR pathway, so that apoptosis results.

## Discussion

Figure 3*B* presents a diagram of the IGF-1-AKT-mTOR pathways and the four points of interaction with p53-regulated genes: *PTEN, TSC2, the β1* and *β2* subunits of *AMPK* and *IGF-BP3*. Previous experiments have shown that a p53 stress response shuts down the mTOR-dependent S6 kinase and activates autophagy, along with p53-mediated apoptosis (22). This communication between these different signal transduction pathways required the p53, PTEN, and TSC2 as shown by a failure of inactivating S6 kinase in cells in which each of these three genes were separately knocked out (22). This inactivation of mTOR activity also required a functional AMPK protein activity as shown by a specific inhibitor of AMPK (22). The results presented here show for the first time that β1 and β2 subunits of *AMPK* are transcriptionally up-regulated in a p53-dependent fashion by a wide variety of stress signals that activate p53 in human (but not mouse) cells. Both ChIP and cloning the

**Table 3.** Gene induction in various tissues of mice ($p53^{+/+}$) after gamma-irradiation

(A)

| Function | Tissue | TSC2 | | PTEN | | IGF-BP3 | | p21 |
|---|---|---|---|---|---|---|---|---|
| | | 8 h | 24 h | 8 h | 24 h | 8 h | 24 h | 8 h |
| Energy metabolism | Skeletal muscle | 2.6 ± 0.6 | 2.0 ± 0.6 | 2.6 ± 0.7 | 2.3 ± 0.8 | 2.5 ± 0.6 | 2.1 ± 0.4 | 3.6 ± 0.3 |
| | Heart | 1.8 ± 0.4 | 2.1 ± 0.6 | 2.1 ± 0.5 | 1.9 ± 0.6 | 1.8 ± 0.3 | 1.6 ± 0.4 | 2.8 ± 0.3 |
| | White fat | 1.4 ± 0.4 | 2.8 ± 0.7 | 1.4 ± 0.6 | 2.7 ± 0.8 | 0.8 ± 0.3 | 1.2 ± 0.3 | 4.6 ± 0.5 |
| | Liver | 1.6 ± 0.2 | 1.8 ± 0.2 | 2.1 ± 0.6 | 2.0 ± 0.3 | 1.2 ± 0.3 | 0.9 ± 0.2 | 8.6 ± 1.3 |
| | Kidney | 1.9 ± 0.4 | 1.5 ± 0.5 | 2.0 ± 0.5 | 1.6 ± 0.5 | 0.9 ± 0.2 | 0.7 ± 0.3 | 12.0 ± 0.9 |
| Others | Spleen | 1.2 ± 0.3 | 1.3 ± 0.4 | 1.2 ± 0.2 | 1.3 ± 0.3 | 2.4 ± 0.3 | 4.1 ± 0.8 | 22.7 ± 1.5 |
| | Thymus | 0.7 ± 0.2 | 1.1 ± 0.4 | 0.7 ± 0.2 | 1.1 ± 0.2 | 4.2 ± 0.8 | 6.6 ± 0.7 | 15.8 ± 1.6 |
| | Small intestine | 0.8 ± 0.2 | 1.1 ± 0.3 | 0.9 ± 0.2 | 1.4 ± 0.4 | 2.8 ± 0.6 | 3.7 ± 0.5 | 4.2 ± 0.6 |
| | Colon | 1.3 ± 0.2 | 1.6 ± 0.4 | 1.4 ± 0.3 | 1.5 ± 0.3 | 0.9 ± 0.3 | 1.6 ± 0.3 | 2.8 ± 0.3 |
| | Lung | 1.7 ± 0.5 | 1.4 ± 0.2 | 2.1 ± 0.6 | 1.5 ± 0.3 | 1.4 ± 0.3 | 2.0 ± 0.4 | 2.9 ± 0.4 |
| | Skin | 0.8 ± 0.2 | 0.9 ± 0.15 | 1.3 ± 0.2 | 1.2 ± 0.15 | 0.8 ± 0.3 | 0.9 ± 0.2 | 2.5 ± 0.3 |
| | Brain cortex | 1.3 ± 0.3 | 1.1 ± 0.2 | 1.3 ± 0.2 | 1.1 ± 0.2 | 1.8 ± 0.4 | 1.2 ± 0.3 | 3.5 ± 0.5 |

(B)

| Function | Tissue | MDM2 | | Pirh2 | | Cop1 | | Cyclin $G_1$ | | Wip1 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 8 h | 24 h | 8 h | 24 h | 8 h | 24 h | 8 h | 24 h | 8 h | 24 h |
| Energy metabolism | Skeletal muscle | 3.0 ± 0.7 | 2.5 ± 1.0 | 2.4 ± 0.6 | 3.0 ± 0.7 | 1.2 ± 0.3 | 1.1 ± 0.2 | 4.8 ± 1.3 | 3.6 ± 0.7 | 2.7 ± 0.8 | 2.1 ± 0.4 |
| | Heart | 3.2 ± 0.4 | 2.6 ± 0.4 | 2.0 ± 0.4 | 2.2 ± 0.5 | 1.3 ± 0.3 | 1.4 ± 0.2 | 1.8 ± 0.5 | 2.1 ± 0.4 | 1.3 ± 0.2 | 1.3 ± 0.4 |
| | White fat | 3.1 ± 0.7 | 2.6 ± 0.8 | 2.2 ± 0.6 | 2.6 ± 0.8 | 1.2 ± 0.3 | 1.3 ± 0.5 | 4.6 ± 0.8 | 4.2 ± 1.2 | 1.5 ± 0.4 | 1.2 ± 0.3 |
| | Liver | 3.6 ± 0.5 | 2.8 ± 0.4 | 2.6 ± 0.3 | 2.7 ± 0.4 | 1.4 ± 0.3 | 1.6 ± 0.4 | 5.3 ± 1.2 | 2.3 ± 0.7 | 2.8 ± 0.9 | 1.4 ± 0.5 |
| | Kidney | 2.8 ± 0.4 | 2.6 ± 0.6 | 2.6 ± 0.5 | 2.1 ± 0.5 | 1.3 ± 0.2 | 1.5 ± 0.6 | 4.5 ± 0.9 | 2.4 ± 0.4 | 2.2 ± 0.6 | 1.2 ± 0.3 |
| Others | Spleen | 6.8 ± 1.1 | 4.8 ± 0.8 | 0.8 ± 0.3 | 0.8 ± 0.2 | 0.6 ± 0.3 | 0.7 ± 0.4 | 6.5 ± 1.8 | 5.7 ± 1.4 | 2.3 ± 0.4 | 1.8 ± 0.3 |
| | Thymus | 4.5 ± 0.9 | 3.6 ± 0.7 | 0.9 ± 0.2 | 1.2 ± 0.3 | 0.8 ± 0.2 | 0.6 ± 0.2 | 4.2 ± 0.8 | 3.6 ± 0.7 | 1.3 ± 0.4 | 0.8 ± 0.3 |
| | Small intestine | 2.1 ± 0.3 | 2.2 ± 0.5 | 0.8 ± 0.2 | 1.3 ± 0.3 | 1.4 ± 0.5 | 1.1 ± 0.3 | 2.3 ± 0.4 | 1.8 ± 0.4 | 1.2 ± 0.3 | 1.6 ± 0.5 |
| | Colon | 2.8 ± 0.8 | 2.3 ± 0.8 | 1.3 ± 0.3 | 1.5 ± 0.4 | 1.3 ± 0.4 | 0.9 ± 0.3 | 5.2 ± 0.9 | 4.7 ± 1.1 | 2.5 ± 0.7 | 2.8 ± 0.8 |
| | Lung | 3.2 ± 1 | 2.4 ± 0.4 | 2.0 ± 0.7 | 1.2 ± 0.2 | 1.3 ± 0.5 | 1.2 ± 0.2 | 5.6 ± 0.8 | 3.3 ± 0.6 | 2.5 ± 0.6 | 1.4 ± 0.3 |
| | Skin | 2.1 ± 0.4 | 2.3 ± 0.5 | 1.5 ± 0.3 | 1.7 ± 0.3 | 0.8 ± 0.3 | 1.3 ± 0.2 | 1.8 ± 0.4 | 2.0 ± 0.5 | 2.0 ± 0.4 | 1.2 ± 0.4 |
| | Brain cortex | 2.0 ± 0.3 | 1.8 ± 0.2 | 1.3 ± 0.2 | 1.4 ± 0.2 | 0.8 ± 0.2 | 1.1 ± 0.2 | 2.5 ± 0.6 | 1.7 ± 0.4 | 1.3 ± 0.3 | 0.9 ± 0.3 |

NOTE: C57BL/6 $p53^{+/+}$ mice were treated with 5 Gy gamma-irradiation and sacrificed at 8 and 24 h postirradiation. The RNA levels of different genes were determined by TaqMan quantitative real-time PCR. All expression levels were normalized to actin. Gene induction was calculated as gene expression levels in irradiated mice compared with nonirradiated mice. No clear induction was observed for AMPK β1 in any tissues.

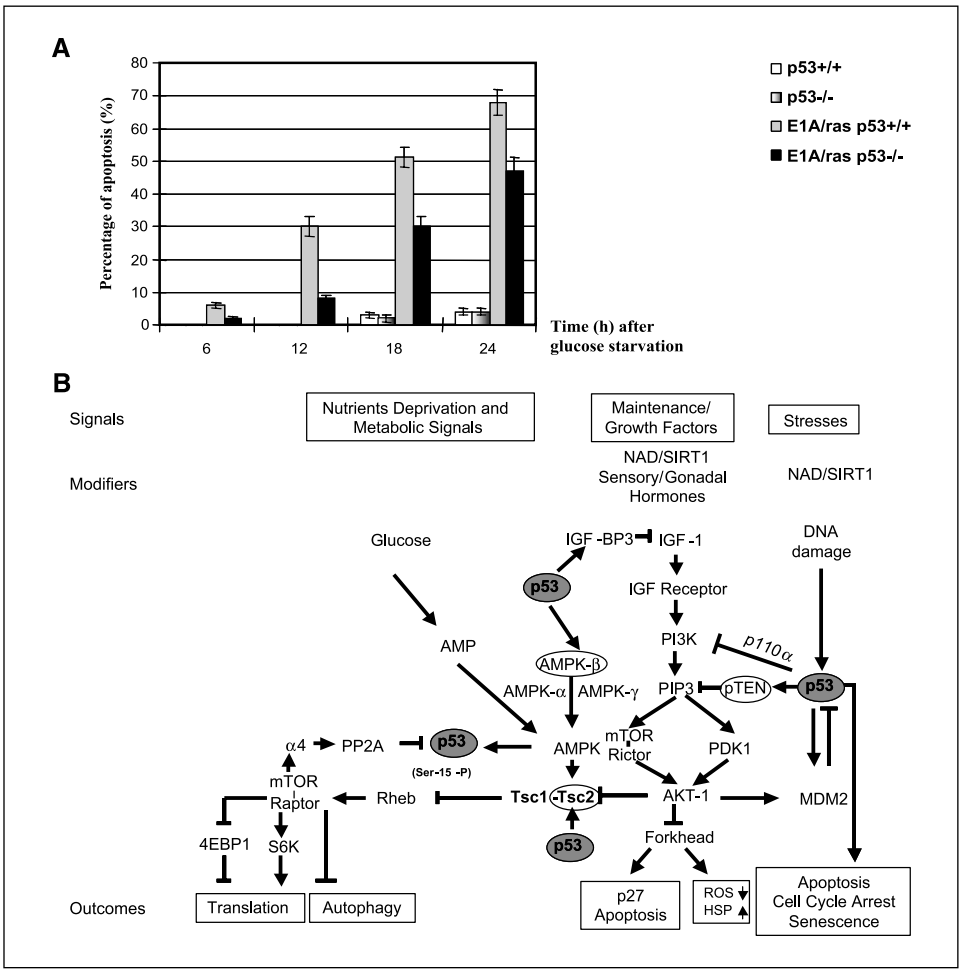



**Figure 3.** The coordinate communication between the p53 and IGF-1-AKT-mTOR pathways. *A,* glucose starvation induces p53-dependent apoptosis in E1A/ras-transformed MEF cells. E1A/ras-transformed MEF $p53^{+/+}$ and $p53^{-/-}$ cells were cultured in complete medium for 24 h before being switched to medium without glucose. Apoptosis was detected at the indicated time points after glucose starvation by Annexin V staining and flow cytometry analysis. *B,* a diagram of the coordinate communication between the p53 pathway and IGF-1-AKT-mTOR pathways.

p53-consensus binding DNA sequences in the *AMPK β1* gene show the essential role of p53 in regulating this gene. A previous report had showed that some stress signals could induce the β1 subunit gene transcription even in the absence of p53 (34). The cell type and stress specificity of this p53 regulation may have led to this limited conclusion. This is an important result because previous experiments had shown that AMPK was essential for p53-mediated regulation of mTOR activity, but the mechanism which mediated this was unclear (22). Similar experiments were employed in this article to show that the p53 protein regulates the *TSC2* gene. In addition, a variety of results presented here confirmed that the *PTEN* and *IGF-BP3* genes were p53-regulated. All four of these gene products serve to negatively regulate the IGF-1-AKT-mTOR pathways in response to stress signals that would otherwise induce errors into DNA replication and cell division (Fig. 3*B*).

The shutting down of the IGF-1-AKT-mTOR pathways by p53 only occurs with a subset of the stress stimuli that activate the p53 protein, pathway, and response. Slowing ribosomal biogenesis (actinomycin D) and DNA damage (Adriamycin) induced the mRNA synthesis of *PTEN, TSC2,* and *AMPK β* subunit, along with the *MDM2* gene, whereas taxol and ALLN regulated the *MDM2* gene but not the other three genes. UV exposure activated the *MDM2* and *AMPK β* subunit, but failed to activate the *PTEN* and TSC2 gene. Clearly, the genes in the p53 pathway have stress-specific responses. Similarly, these genes show cell type– and tissue type–specific responses both *in vitro* (cell culture) and *in vivo* (mouse). Perhaps this is most dramatically illustrated by the observation that the *TSC2* and *PTEN* genes were transcriptionally activated by stress through a p53 response (not in $p53^{-/-}$ mice) in skeletal muscle, heart, white fat, liver, and to a lower extent, the kidney, which are the tissues where the IGF-1-mTOR pathways function to process insulin-dependent glucose utilization to generate energy using oxidative phosphorylation (30). The IGF-BP3–secreted protein was regulated by a stress-induced p53 response in spleen, thymus, skeletal muscle, heart, and the small intestine. *MDM2* is regulated by p53 in most of these tissues, whereas *p21* is p53 controlled in all of these tissues. These studies have uncovered three classes of p53 regulated genes: (*a*) genes that are induced to synthesize mRNA in all tissue types after a stress signal (*p21, MDM2,* and *cyclin* $G_1$); (*b*) genes that are regulated by p53 in tissues that rely on insulin-regulated glucose metabolism for maximal energy production (*TSC2* and *PTEN*); and (*c*) genes which respond to p53-mediated stress signals in only selected tissues *(IGF-BP3, Pirh2,* and *Wip1*). This distribution of stress signals and the tissue specificity seems to have functional consequences associated with it. It may determine which cell or tissue type shuts down the IGF-1-mTOR pathways after a stress signal.

Glucose starvation signals through the LKB-1 and AMPK kinases to phosphorylate and activate the TSC1/2 complex, which turns down the mTOR activity (Fig. 3*B*). mTOR also phosphorylates and activates the α-4 subunit of the PP2A phosphatase, which acts to remove a phosphate group from p53 $Ser^{15}$. The AMPK adds a

phosphate group to p53 $Ser^{15}$ and the failure to remove it by the α-4-PP2A phosphatase during glucose starvation (22, 23) has been observed experimentally (Fig. 3*B*). When these events transpire in normal MEF, the p53 protein, although phosphorylated by the AMPK, is not stabilized or activated and the cell eventually slows its growth rate due to glucose starvation. When this same experiment is carried out in E1A-transformed MEF, p53 is stabilized and activated and the cells die of a p53-mediated apoptosis. In this case, the E1A protein binds to the Rb protein, which frees the E2F-1 transcription factor. E2F-1 transcribes the p19 ARF gene to inhibit MDM2 activity (45, 46). The p53 levels increase and these cells undergo apoptosis. This is a classical p53-positive feedback loop between loss of Rb function and activation of p53, which senses and titrates the presence of activated oncogenes (E1A, myc, ras, β-catenin, etc.) in cells and informs the p53 pathway to focus on apoptosis as an outcome. In this case, glucose deprivation is the stress signal, sensed in the presence of an activated oncogene, which is communicated to the p53 pathway via AMPK and LKB-1. Interestingly, a second positive feedback loop is present in these pathways (ref. 47; Fig. 3*B*). A p53 stress signal will induce PTEN expression in some cell types, which inactivates AKT-1 by reducing the PIP-3 levels and lowers MDM2 activity and increases p53 activity. This sets up a positive feedback loop, raising both PTEN and p53 activity in selected cell types and leading to apoptosis or cell cycle arrest. In other cell types (such as prostate tissues or fibroblasts in which p53 does not regulate PTEN in this fashion), the loss of PTEN activity results in the activation of p53 and the initiation of a p53-mediated senescence (48). The mechanisms and pathways that mediate this effect remain obscure. For this reason, *PTEN* knockout fibroblasts (that are eventually selected to grow and survive in culture) have no detectable p53 activity (49). Similarly, in a mouse prostate tissue, a *PTEN* knockout triggers a nonlethal prostate tumor after a long latency (kept in check by p53), whereas a PTEN and p53 double knockout develops prostate cancer rapidly (in 2 weeks) and is lethal (48). Thus, PTEN and p53 clearly talk to each other in different ways in different tissues, suggesting that the diagrams of signal transduction pathways (like Fig. 3*B*) have a strong tissue-specific (and perhaps stress-specific) component. The long-term goal is to be able to draw and understand these diagrams for each tissue type and each cancer.

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