Supplementary Materials and Methods

Primary antibodies used in this study:

anti-p53 mouse monoclonal antibody (DO-1) (Calbiochem)

anti-p53 goat polyclonal antibody（FL393）#sc-6243 (Santa Cruz Biotechnology)

anti-phospho p53 (Ser46) rabbit polyclonal antibody #2521 (Cell Signaling Technology)

anti-PLK2 mouse monoclonal antibody (E-10) #sc-374643 (Santa Cruz Biotechnology)

anti-PTP4A1 mouse monoclonal antibody #sc-271879 (Santa Cruz Biotechnology)

anti-cleaved caspase3 rabbit polyclonal antibody #9661 (Cell Signaling Technology)

anti-PARP rabbit polyclonal antibody #9542 (Cell Signaling Technology）

anti-β-actin mouse monoclonal antibody (SIGMA)

anti-actin mouse monoclonal antibody, clone C4 (Merck Millipore)

anti-GAPDH antibody sc-25778 (Santa Cruz Biotechnology)

Secondary antibodies used in this study:

anti-goat IgG HRP - linked Whole antibody #sc-2350 (Santa Cruz Biotechnology)

anti-rabbit IgG, HRP - linked Whole antibody #NA931V (GE Healthcare)

anti-mouse IgG, HRP - linked Whole antibody # NA934V (GE Healthcare)

Immunofluorescence

H1299 cells were transfected with the indicated expression vector plasmid, and harvested 24 hrs post-transfection. Cells were fixed with 4 % paraformaldehyde in PBS for 10 min, and permeabilized with 0.2 % Triton X-100 in PBST for 2 min. Cells were blocked with BSA (5 mg/ml) and 50 mM Glycine in PBST for 1 hr. The cells were sequentially incubated with anti-Flag antibody for 1 hr at room temperature and AlexaFluor 594-labeled secondary antibody (Molecular Probes, Eugene, OR, USA), and mounted with DAPI (2.5 μg/ml) in DABCO glycerol. ER was visualized using FITC-ER stain (CHEMICON #SC-100). Images were obtained by using a Keyence BZ-9000 fluorescence microscope.

Plasmids and transfection

p53 constructs: each p53 construct was cloned in pcDNA3 or pMX vector as described [1]. Transient transfection assays were performed using Lipofectamine Plus reagent

(Life Technologies) or Lipofectamine 2000 transfection reagent (Invitrogen). siRNAs were introduced using RNAiMAX (Invitrogen, Carlsbad, CA). Non-Targeting Control pool (D-001810-10-50), ON-target plus Human TP53-targeting (L-003329-00), Human PTP4A1-targeting (L-006333-00), and human PLK2-targeting (L-003325-00) ON-TARGET plus SMART pool siRNAs were purchased from Thermo Scientific Dharmacon.

Cell culture and establishment of stable cell lines

Cell culture was performed as described [2]. Where indicated, cells were treated with thapsigargin (Sigma-Aldrich). Stable cell lines expressing temperature-sensitive FL-p53 and Δ1stTAD-p53 were generated as described [1]. Stable transfectants were obtained by infecting cells with recombinant lentiviruses. In each case, as the control cell line, cells were also infected with empty lentiviruses expressing only the drug resistance gene. Infection was performed in the presence of 4 μg/ml polybrene (Sigma). The shRNA targeting p53 contains a 19-nt sequence derived from the ORF of human p53 (nucleotides 775-793). Cells were selected in 0.6 mg/ml hygromycin (WAKO). All cells lines were kept polyclonal rather than monoclonal to avoid the variation often encountered with single clones.

Microarray expression analysis, ChIP-chip and ChIP sequence

Microarray expression analysis, ChIP-chip and ChIP sequence was performed as described [3-5, 7, 8]. To detect binding of Δ1stTAD-p53, HCT116 *p53* -/- cells were subjected to FBS starvation for 60 hrs. For FL-p53 induction, HCT116 *p53* +/+ cells were treated with 5-FU (0.375 mM, 9 hrs). Antibodies against p53 (FL393, Santa Cruz), H3K27ac, H3K4me1, H3K4me3 (07-473, Millipore, ChIP grade) or phospho-RNAP II were used to precipitate immune complexes. Antibodies against H3K27ac, H3K4me1 and phospho-RNAP II were kindly provided by Dr. Hiroshi Kimura, Tokyo Institute of Technology. The antibodies have been used in previous studies for ChIP and ChIP-seq analysis [3-8]. Cells were cross-linked with 1% formaldehyde for 10 min, followed by quenching with 125 mM glycine for 5 min. DNA fragmentation was performed by using UD 201 (Tomy, Tokyo, Japan) with the following settings: output level 2, 50% duty, 15 sec, 5 cycles, on floating ice to obtain 200–500 bp DNA fragments. The fragmented samples were incubated with antibodies bound to protein

A-sepharose beads (GE Healthcare) or Dynabeads (Life technologies) at 4 °C overnight. The beads were then washed eight times and eluted with elution buffer. The eluates and input were treated with pronase at 42°C for 2 hrs and then underwent incubation at 65°C overnight to reverse the cross-linking. The immunoprecipitated and input DNA were purified by 2 extractions with phenol:chloroform, followed by ethanol precipitation. Purified DNA samples were resuspended in 20 µL of Tris-HCL (pH 8.0). The concentration of the DNA sample was measured with a Qubit fluorometer (Life Technologies). The ChIP DNA was used to prepare library samples following the manufacturer's instructions (Illumina). Deep sequencing was performed on the Solexa Genome Analyzer II to obtain 36-bp single reads.

Sequencing tags were mapped to the build #36 reference human genome using ELAND. Total sequenced reads for p53 (FBS starvation 60h and 5-FU 9h), H3K4me3 (5-FU 0, 9h), H3K4me1 (5-FU 0, 9h), H3K27ac (5-FU 0, 9h) and phospho-RNAP II (5-FU 0, 9h) were 26730379, 17020836, 22559531, 8296440, 9319944, 29174994, 27258981, 15964489 and 15142757, respectively. ChIP signal values (ChIP counts / estimated or input counts) were generated as previously reported [9]. Probability values were generated as all fragments were randomly mapped to the non-repetitive sequences of the Human Genome. Analyzed files for the genome browser (IGB) were generated by calculating ChIP signal values. Peak-calling for p53 was performed using MACS (http://liulab.dfci.harvard.edu/MACS/). Motif analysis at p53-binding peaks was performed by using HOMER software (http://homer.salk.edu/homer/index.html). Data were analyzed using the MACS14 (MACS ver 1.4.2) software to identify genes having a threshold of >20 fold enrichment against background. The binding sites were also filtered by $p < 0.00001$ and signal rate > 15. Binding sites were further filtered to identify those within 10 kbps of a transcription start site or gene body using Refgene.txt of UCSC web site (http://hgdownload.soe.ucsc.edu/goldenPath/hg18/database/). The resulting genes were selected as genes bound by FL-p53 or Δ1stTAD-p53. p53 consensus regions were computationally determined using the TRANSFAC database. To evaluate the results of FL-p53 binding sites, we selected and analyzed 2 data sets from ReMAP [10] obtained using HCT116 cells treated with 5-FU (https://www.ncbi.nlm.nih.gov/sra/?term=SRR1409975, [11]) or ionizing radiation (https://www.ncbi.nlm.nih.gov/sra/?term=SRR1539836, [12]), respectively. Base-calling and quality-control was performed using the Illumina pipeline Casava analysis package,

and ChIP-Seq reads were aligned to the human hg18 using the Batman algorithm. Peak calling was done using the MACS 1.4 package. The setting for peak calling was bw=300, mfold=10,30, and a p-value of at least 1e-5. A total of 3,819 and 2,567 peak regions were identified in the ReMAP database for 5FU and IR treatments respectively. Out of these, 3, 136 of 3,819 (82.1%) and 1,011 of 2,567 regions (39.4%) overlapped with our FL-p53 binding sites.

ChIP assay

ChIP assay was performed as described [3]. The control, ts-FL-p53- and ts-Δ1stTAD-p53-expressing Saos2 cell line were tested for p53 binding to PLK2, PTP4A1 and RPS27L promoters upon temperature shift to the permissive temperature. Cells were collected 6 hrs after temperature shift. Prepared cell lysates were immunoprecipitated with EZview Red ANTI-FLAG M2 Affinity Gel (Sigma-Aldrich), and used for subsequent analysis. PCR primers were designed so that the PCR products contain the p53 responsive element in the middle of the product.

Primers:

PLK2-5primer; 5'-TCTTAGAGAGTAGGTTTGCACAAATCTG-3'

PLK2-3' primer; 5'-GAAAAGAGGCTTATTGCCATTAGAGAG-3'.

PTP4A1-5primer; 5'-CAGGCTCCACCTGGTAATTCG-3'

PTP4A1-3' primer; 5'-AGTCCTTCGCGTCAGTGTAAC-3'.

RPS27L-5primer; 5'-GAGTCATATGGGACGGATGAG-3'

RPS27L -3' primer; 5'-CAGCCACCGCCTCTGAATTGCA-3'.

Luciferase reporter assay

Plasmids carrying the p53 responsive elements were generated as follows. PCR products were amplified from genomic DNA using the primers from the ChIP assay and cloned into pGL3 or PicaGene basic vector (WAKO) containing a minimal promoter. Luciferase reporter assays were performed as described [3-5]. For the luciferase reporter assay shown in Fig. 6C, H1299 cells were seeded in 96-well dishes and transduced with control LacZ-expressing (Ad-LacZ) or Δ1stTAD-p53-expressing adenovirus (Ad-Δ1stTAD-p53) at a multiplicity of infection (moi) of 4. Cells were then transfected with 20 ng of a firefly luciferase reporter gene together with 3 ng of a Renilla luciferase expression vector (pGL4 [TK-Rluc] vector, Promega) 6 hrs post-infection as an internal

control for transfection efficiency. For the luciferase reporter assay shown in Figs. 6C-6E, H1299 cells were seeded in 24-well dishes and co-transfected with 33 ng of a firefly luciferase reporter gene and 133 ng of each p53 gene cloned in the pMX vector together with 10 ng of the pGL4 [TK-Rluc] vector. Cells were harvested 24 hrs (Fig. 6C) or 53 hrs (Figs. 6D and 6E) post-transfection, and analyzed using the Dual-Luciferase Reporter Assay System (Promega). All of the luciferase reporter assay data are the mean-fold activation +/- SD of three independent samples.

Construction of recombinant adenovirus expressing Δ1stTAD-p53, PLK2 and PTP4A1

Recombinant adenovirus constructs were made as described [2, 3]. Sequences containing the full open reading frames of PTP4A1 and PLK2 were inserted into the *Smi* I site of the pAxCAwtit vector. The multiplicity of infection was determined as the 50 % tissue culture infectious dose ($TCID_{50}$) in 293 cells, an E1-complementing helper cell line, according to the manufacturer's instructions.

Flow cytometry and cell death assay

To detect cells having a sub-G1 DNA content, cells were collected and fixed with 70 % ethanol overnight. Cells were then washed with PBS and incubated with 10 μg/ml propidium iodide and 100 μg/ml RNase A. To detect TUNEL-positive cells, cells were subjected to TdT-mediated UTP nick end labeling using the In Situ Cell Death Detection Kit, Fluorescein (Roche Diagnostics). Flow cytometry analysis was performed using a FACS Calibur (Becton Dickinson).

Real-time PCR

For the detection of PLK2 (Hs01573405g1) and PTP4A1 (Hs00743856s1), a TaqMan probe from Applied Biosystems were used. For the detection of PHLDA3 (Hs.PT.56a.14572547.g) and RPS27L (Hs.PT.58.26134760), a PrimeTime qPCR Assay from Integrated DNA Technologies or TB Green Premix Ex Taq (Tli RNaseH Plus) (TaKaRa) were used. To detect GAPDH, p53 and PTP4A1, custom-designed TaqMan Dual-Labeled Probes from Sigma-Aldrich were also used.

GAPDH Forward: CTCCTCTGACTTCAACAGCGA

GAPDH Reverse: CCAAATTCGTTGTCATACCAG

GAPDH Probe: [FAM] CCCACTCCTCCACCTTTGACGCTGG [TAMRA]

TP53 Forward: GGAACTCAAGGATGCCCAGG

TP53 Reverse: ATGGCGGGAGGTAGACTGAC

TP53 Probe: [FAM] AGCAGGGCTCACCTCCAGCCAGCCACCTG [TAMRA]

PTP4A1 Forward: ATTGAAGGTGGAATGAAATACGAAG

PTP4A1 Reverse: TACTTCTCCAAATACAGAAGTTGCT

PTP4A1 probe: [FAM] AGCTCCACGCCGCTTTTGTCTTATGAATTG [TAMRA]

RPS27L Primer 1: CTGTTTGAATCATTAGTGTTGCTTTC

RPS27L Primer 2: CAGTGGTTCTTTGTGTAGGTTG

RPS27L Probe: /56-FAM/TCTGTGAGT/ZEN/CTGGCCTTTCCTCCT/3IABkFQ/

PHLDA3 Primer 1: GTCCATGCCTTCCACCTT

PHLDA3 Primer 2: ACCATCTTTCCTTCATGCTACC

PHLDA3 Probe: /56-FAM/TGCCCCCAC /ZEN/AAGCCAGAGG /3IABkFQ/

Supplementary References

[1] Ohki R, Kawase T, Ohta T, Ichikawa H, Taya Y. Dissecting functional roles of p53 N-terminal transactivation domains by microarray expression analysis. Cancer Sci. 2007; 98: 189-200.

[2] Ohki R, Nemoto J, Murasawa H, et al. Reprimo, a new candidate mediator of the p53-mediated cell cycle arrest at the G2 phase. J Biol Chem. 2000; 275: 22627-30.

[3] Kawase T, Ohki R, Shibata T, et al. PH domain-only protein PHLDA3 is a p53-regulated repressor of Akt. Cell. 2009; 136: 535-50.

[4] Ezawa I, Sawai Y, Kawase T, et al. Novel p53 target gene FUCA1 encodes a fucosidase and regulates growth and survival of cancer cells. Cancer Sci. 2016; 107: 734-45.

[5] Asano Y, Kawase T, Okabe A, et al. IER5 generates a novel hypo-phosphorylated active form of HSF1 and contributes to tumorigenesis. Sci Rep. 2016; 6: 19174.

[6] Kimura H, Hayashi-Takanaka Y, Goto Y, Takizawa N, Nozaki N. The organization of histone H3 modifications as revealed by a panel of specific monoclonal antibodies. Cell Struct Funct. 2008; 33: 61-73.

[7] Kawase T, Ichikawa H, Ohta T, et al. p53 target gene AEN is a nuclear exonuclease required for p53-dependent apoptosis. Oncogene. 2008; 27: 3797-810.

[8] Chen Y, Takikawa M, Tsutsumi S, et al. PHLDA1, another PHLDA family protein that inhibits Akt. Cancer Sci. 2018; 109: 3532-42.

[9] Wakabayashi K, Okamura M, Tsutsumi S, et al. The peroxisome proliferator-activated receptor gamma/retinoid X receptor alpha heterodimer targets the histone modification enzyme PR-Set7/Setd8 gene and regulates adipogenesis through a positive feedback loop. Molecular and cellular biology. 2009; 29: 3544-55.

[10] Cheneby J, Gheorghe M, Artufel M, Mathelier A, Ballester B. ReMap 2018: an updated atlas of regulatory regions from an integrative analysis of DNA-binding ChIP-seq experiments. Nucleic Acids Res. 2018; 46: D267-D75.

[11] Sanchez Y, Segura V, Marin-Bejar O, et al. Genome-wide analysis of the human p53 transcriptional network unveils a lncRNA tumour suppressor signature. Nat Commun. 2014; 5: 5812.

[12] Desantis A, Bruno T, Catena V, et al. Che-1 modulates the decision between cell cycle arrest and apoptosis by its binding to p53. Cell Death Dis. 2015; 6: e1764.