Oncogene (2005) 24, 1461–1466
© 2005 Nature Publishing Group All rights reserved 0950-9232/05 $30.00
www.nature.com/onc

dapk1, encoding an activator of a p19^{ARF}-p53-mediated apoptotic checkpoint, is a transcription target of p53

Alain Martoriati[1,2,5], Gilles Doumont[1,4,5], Myriam Alcalay[3,4], Eric Bellefroid[2], Pier Giuseppe Pelicci[3,4] and Jean-Christophe Marine*[,1]

[1]*Laboratory For Molecular Cancer Biology, Flanders Interuniversity Institute for Biotechnology (VIB), Technologiepark, 927, Ghent B-9052, Belgium;* [2]*Unit of Molecular Embryology, Free University of Brussels (ULB-IBMM), Gosselies, Belgium;* [3]*Department of Experimental Oncology, European Institute of Oncology, 435 Via Ripamonti, Milan 20141, Italy;* [4]*FIRC Institute of Molecular Oncology, Via Adamello, 16, Milan 20139, Italy*

The p53 tumour suppressor functions as a transcriptional activator, and several p53-inducible genes that play a critical proapoptotic role have been described. Moreover, p53 regulates the expression of various proteins participating in autoregulatory feedback loops, including proteins that negatively control p53 stability (Mdm2 and Pirh2) or modulate stress-induced phosphorylation of p53 on Ser-46 (p53DINP1 or Wip1), a key event for p53-induced apoptosis. Here, we describe a new systematic analysis of p53 targets using oligonucleotide chips, and report the identification of *dapk1* as a novel p53 target. We demonstrate that *dapk1* mRNA levels increase in a p53-dependent manner in various cellular settings. Both human and mouse *dapk1* genomic loci contain DNA sequences that bind p53 *in vitro* and *in vivo*. Since *dapk1* encodes a serine/threonine kinase previously shown to suppress oncogene-induced transformation by activating a p19^{ARF}/p53-dependent apoptotic checkpoint, our results suggest that Dapk1 participates in a new positive feedback loop controlling p53 activation and apoptosis.
Oncogene (2005) **24,** 1461–1466. doi:10.1038/sj.onc.1208256
Published online 20 December 2004

Keywords: apoptosis; *dapk1*; p53; Mdm4

p53 prevents cancer development by inhibiting the proliferation of stressed or abnormal cells (Levine, 1997). p53 functions as a sequence-specific DNA-binding transcription factor that promotes antiproliferative responses, including cell-cycle checkpoints, cellular senescence and apoptosis (Lane, 1992). The precise nature of the p53 transcriptional programs and the complex mechanisms that decide whether or not a cell dies in response to p53 remain elusive (Vousden and Lu, 2002). Understanding these mechanisms is of particular interest, since the ability of p53 to induce apoptosis is thought to be central to its tumour suppressor activity (Schmitt *et al*., 2002).

p53 activates the expression of numerous genes, including genes encoding for proteins that have a purported activity in triggering apoptosis (El-Deiry, 1998), such as the mitochondrial proteins Bax, Noxa, Puma, BID, the apoptosis protease-activating factor-1 (Apaf-1), p53AIP1 and proteins that play a role in death receptor-mediated apoptosis, such as Fas, KILLER/DR5 or PIDD. Even if a full p53-dependent apoptotic response requires the coordinated induction of all or some of these target genes, Puma, and to a lesser extent NoxA, are critical mediators of p53-induced cell death (Jeffers *et al*., 2003; Villunger *et al*., 2003). p21/Waf1/Cip1, another well-studied p53-target genes, mediates at least in part p53-induced G1 arrest. Among the many p53 transcriptional targets identified so far are several mediators of feedback loops for p53. Mdm2 and Pirh2 are p53-target genes encoding E3 ligase proteins that contain a Ring-H2 domain and target p53 for ubiquitin-dependent proteolysis (Barak *et al*., 1993; Haupt *et al*., 1997; Leng *et al*., 2003). In addition to its favouring proteolysis, Mdm2 directly inhibits the transcriptional activity of p53. *wip1* and *p53DINP1* are other known p53-inducible genes encoding for proteins able to modulate p53 biological activities (Fiscella *et al*., 1997; Takekawa *et al*., 2000; Okamura *et al*., 2001).

Since p53 is such a potent inhibitor of cell growth, restraint of p53 activity during normal growth and development is of paramount importance. Central to this process are the Mdm2- and Mdm4- (or Mdmx) related proteins (Marine and Jochemsen, 2004). *mdm4*-null embryos die at midgestational stages exhibiting p53-dependent apoptosis in the developing neuroepithelium (Neur) and an overall growth deficiency, especially in the foetal liver. The latter is likely due to arrest of the mutant cells in the G1 phase of the cell cycle and, at least partly, induction of p21 expression (Migliorini *et al*., 2002). As the Mdm4/p53 interaction is critical for cell-cycle progression and survival, we used this mouse model to search for new key downstream effectors of p53-dependent cell-cycle arrest and apoptosis. To this end, we monitored changes in gene expression in the Neur and in nonapoptotic tissues, including the foetal

*Correspondence: J-C Marine; E-mail: chris.marine@dmbr.ugent.be
[5]These authors contributed equally to this work
Received 2 June 2004; revised 1 September 2004; accepted 5 October 2004; published online 20 December 2004

liver (N. Neur), of Mdm4-mutant embryos using oligonucleotide microarrays (Alcalay *et al*., 2003). Our analyses revealed significant changes in the expression of 400 and 845 genes in *mdm4*-deficient Neur and non-neuroepithelial tissues, respectively. Among them, 113 genes (equivalent to 30% of those regulated in Neur and 14% of those regulated in non-neuroepithelial tissues) were concordantly regulated in both histological compartments. Expression of 213 and 274 genes (92 in common) was increased in the Neur and the rest of the *mdm4*-null embryos, respectively. Microarray results have been deposited in the ArrayExpress database at EBI (E-MEXP-155). Detailed description of the methods and data analysis, and the lists of regulated genes can be obtained from our website http://www.dmbr.ugent.be/lmcb/contents/p53targets/ (see Supplementary Methods).

More than 20 known p53-responsive genes were identified among the regulated genes of both cellular compartments, including p21, Mdm2, CyclinG, p53DINP1, NoxA, Bax, Apaf-1, Perp and Pig1, thereby confirming the rationale of our experimental approach. Intriguingly, with the exception of the Fas ligand, known p53 proapoptotic target genes such as NoxA, Perp, Apaf-1 and to a lesser extent Bax were found upregulated not only in the Neur but also in the non-neural tissues, which did not exhibit signs of apoptosis. Quantitative PCR (Q-PCR) analyses showed no expression of the neural-specific marker Nestin in the non-neural samples excluding their contamination by apoptotic neural cells (not shown). It appears, therefore, that increased p53 transcriptional activity in the *mdm4*-mutant embryos results in the stimulation of the same set of target genes in all tissues and that engagement of the apoptotic pathway is cell type-specific and p53-independent.

This report focuses on *dapk1*, which encodes a proapoptotic serine/threonine kinase that was found upregulated in *mdm4*-null embryos. Semiquantitative PCR (Figure 1a) and nonradioactive *in situ* hybridization (ISH) confirmed induction of *dapk1* expression in *mdm4*-mutant embryos (Figure 1b). Levels of *dapk1* induction (from two- to four-fold) were comparable to those of other known p53-proapoptotic targets, such as Apaf-1, Bax and PUMA (data not shown).

We next determined Dapk1 expression levels after endogenous p53 activation in a series of murine cellular systems by semiquantitative and Q-PCR. Activation of p53 by induction of a conditional allele of $p19^{ARF}$ in NIH-3T3 cells (pMTArf cells; Kuo *et al*., 2003) led to increased *dapk1* expression (Figure 2a). *dapk1* mRNA was induced in response to DNA damage such as following UV-irradiation (IR) (Figure 2b), doxorubicin treatment and gamma–IR (Figure 2c) of wild-type, but not p53-null, mouse embryonic fibroblasts (MEFs). Similarly, we observed induction of *dapk1* mRNA in response to doxorubicin treatment in wild-type, but not p53-null, embryonic stem cells (ES) (Figure 2d). Notably, in both of these two primary cellular systems (MEFs and ES), *dapk1* basal mRNA levels did not depend on the presence of p53. Finally, induction of

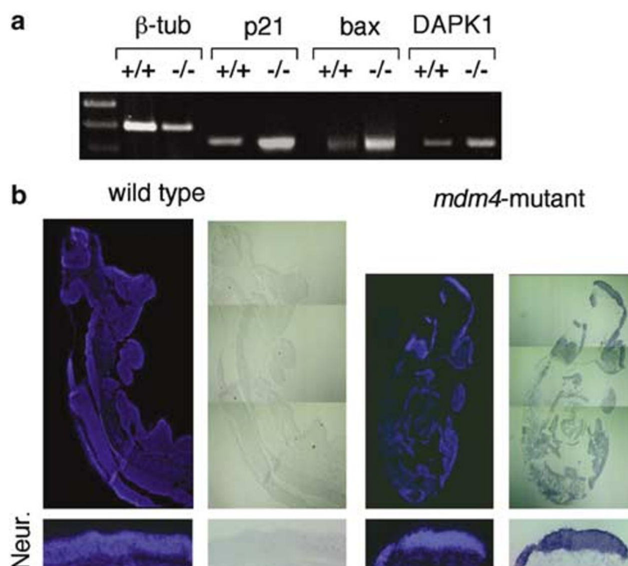

Figure 1 Increased *dapk1* expression in *mdm4*-mutant embryos. (**a**) Semiquantitative PCR expression analysis revealed increased *dapk1* mRNA levels in *mdm4*-homozygous mutant embryos (−/−) compared to wild-type embryos (+/+). Expression of p21 and bax, well-known p53 targets, is also increased. β-tubulin (β-tub) served as a normalization control. Total RNA was prepared using RNeasy miniKit (Qiagen) according to the manufacturer's protocol. Total RNA (1 μg) was reverse transcribed using a SuperScript kit (Invitrogen). PCR primers were as follows: *p21*, sense 5′-GGAGCAAAGTGTGCCGTTGTC-3′; antisense 5′-GAGGAAGTACTGGGCCTCTTG-3′; *Bax*, sense 5′-GGCGAATT GGAGATGAACTGG-3′; antisense 5′-GCTAGCAAAGTAGAA GAGGGC-3′; *mDAPK1*, sense 5′-TTGCACAACAGCTACACAGC-3′; antisense 5′-ATAGTC CCACTACTCAGGTC-3′; *β-tubulin* sense, 5′CAACGTCAAGACGGCCGTGT G-3′, antisense: 5′-GACAGAGGCAAACTGAGCACC-3′. (**b**) Nonradioactive ISH on E10.5 embryo paraffin sections shows no expression of *dapk1* in wild-type and ubiquitous expression in *mdm4*-mutant embryos, particularly high in the Neur. ISH was performed using digoxygenin-labelled antisense RNA probes. The *dapk1* probe was generated using linearized pCMVSPORT6-DAPK1 as a template (EST BG295189, Image Consortium)

dapk1 mRNA level was confirmed at the protein level (Figure 2e) using the human osteosarcoma SAOS-2-p53 cell line, after the expression of a conditional p53 allele (Nakano and Vousden, 2001). Together, these results demonstrate that activation of p53 in normal or tumour cells leads to the activation of *dapk1* expression. The kinetic of this induction was similar to that of several known p53-inducible target genes, such as p21 (Figures 2a, b and d), suggesting that *dapk1* is a direct p53 transcriptional target.

Other well-characterized p53-responsive genes contain p53-binding sites either upstream of the first exon or within the first intron. Sequence analysis of the same region of human and murine *dapk1* revealed the presence of several putative p53-binding sites, in the first intron. Furthermore, the p53MH algorithm (Hoh *et al*., 2002) predicted additional putative p53-binding sites (including perfect-match consensus sequences) in both mouse and human *dapk1* loci (Figure 3a). To determine whether p53 binds to the candidate response elements in the mouse and human *dapk1* first intron, we

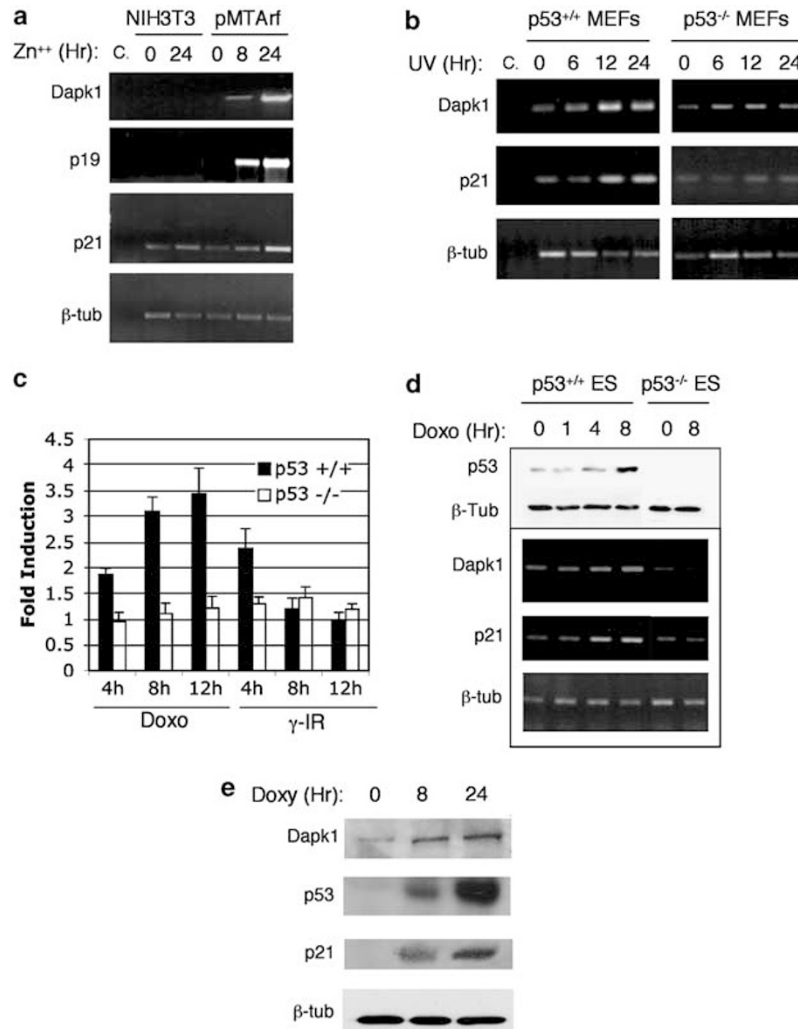

Figure 2 *dapk1* expression is p53 inducible. SAOS-2 (p53-inducible), NIH-3T3 or pMTArf and phoenix packaging cell lines were grown in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum, 2 mM glutamine and 100U/ml penicillin and streptomycin. MEFs were prepared from E12.5 wild-type and $p53^{-/-}$ embryos and cultured as described (15). (**a**) Semiquantitative PCR analysis shows expression of *dapk1* in p19-ARF-inducible pMTArf cells exposed to $ZnSO_4$ (100 μM, 17) for 8 and 24 h. Expression of p19ARF, p21 and dapk1 was detected only in the pMTArf cells and not in the NIH-3T3 parental cell line. β-tub served here as a normalization control. See Figure 1a. *p19* PCR primers were: sense 5′-AGGGATCCTTGGTCACTGTGAGGATTC-3′; antisense: 5′-GCAAAGCTTGAGGCCGGATTTAGCTCTGCT-3′. (**b**) Semiquantitative PCR analysis shows *dapk1* expression in p53 wild-type or p53-deficient MEFs exposed to UV (30 J/cm^2). Induction of p21 and dapk1 expression was detected only in wild-type cells and not in p53-deficient cells. β-tub served here as a normalization control. (**c**) Q-PCR analysis shows *dapk1* expression in p53 wild-type or p53-deficient MEFs exposed to doxorubicin (Doxo, 0.2 μg/ml) and γ-IR (8 Gy). Induction of expression of *dapk1* was only evident in wild-type cells and not in p53-deficient cells. These assays were performed following the manufacturer's specifications (PE Applied Biosystems). Primer pairs and TaqMan probes were designed by Applied Biosystems (assays on demand). Each sample was analysed in triplicate. (**d**) Western analysis (top panel) and semiquantitative PCR (lower panel) shows the expression of p53 protein levels and *dapk1* mRNA levels, respectively, in p53 wild-type or p53-deficient ES cells exposed to doxorubicin (Doxo, 0.2 μg/ml). Induction of p21 and dapk1 mRNA was concomitant with p53 protein stabilization and was detected only in wild-type cells. β-tub served here as a normalization control. (**e**) Western blot showing elevated p53, p21 and Dapk1 protein levels in p53-inducible SAOS-2 cells following doxycycline (Doxy) treatment. Western analyses were performed as described (15). The primary antibodies used were: polyclonal anti-p53 (FL-393, Santa Cruz), monoclonal anti-p21 (F-5, Santa Cruz), monoclonal anti-β-tubulin (Sigma) and anti-Dapk1 (Sigma)

first carried out band shift assays comparing the binding activities of double-stranded oligonucleotides containing *dapk1*-derived sequences and well-characterized p53-binding sites (PG13) as control (not shown). Of four mouse and two human sites tested, only one (p53RE2) efficiently bound p53 after activation of its DNA-binding function with the antibody pAb421 (Figure 3b). Oligonucleotides with binding sites for an unrelated transcription factor (suppressor of hairless, SuH) or mutant p53RE2-binding sites showed no p53-binding activity (Figure 3b). Binding to both mouse and human p53RE2 was efficiently competed by excess of unlabelled p53RE2 but not by oligonucleotides harbouring unrelated binding site or mutant p53RE2-binding sites

Figure 3 Identification of a p53-binding site in dapk1 intron 1. (**a**) Partial genomic structure of mouse *dapk1* showing exon–intron organization at the 5′-end of the locus. Nine potential p53-binding sites were identified (RE1–RE9) in this region. The distance between each site and a nucleotide of reference (0, chosen 10 kb upstream to the first coding exon) is indicated. The complete sequence of RE2 is compared to the consensus p53-binding site. The boxed REs were tested for their ability to bind p53 in gel shift assays. (*) Sites identified by the algorithm described by Hoh *et al.* (2002). (**b**) DNA-binding activity of p53 to oligonucleotides encoding the potential p53-binding sites (p53-responsive element, p53RE2) located in the *dapk1* locus (*mDAPK1*) (5′-GGGCATGTCCCTGACTGGCCC-3′) and the human locus (*hDAPK1*) (5′-GAACTTGTTCTAATGGCATGTCT-3′), and the p53 consensus binding site (PG13: 5′-GGGCAAGCCCGGGCAAGCCC-3′). Oligonucleotides were ^{32}P end-labelled with T4 kinase ($10\,\mu/\mu$l, Invitrogen) and hybridized with the complementary oligonucleotide. Probes were incubated for 15 min with an *in vitro* transcribed and translated p53 protein (Promega) and the C-terminal mouse monoclonal antibody pAb421 (Oncogene) allowing p53 binding to DNA. The DNA–protein complexes were separated from the nonbound probes by electrophoresis. Competitive inhibition of p53 binding to RE2 was observed with unlabelled cold RE2, but not with unlabelled unrelated suppressor of hairless-responsive element, SuH RE, when added at $10\times$, $50\times$ and $100\times$ molar excess over the labelled RE2 oligonucleotide. (**c**) ChIp assays of p53 DNA-binding activity in zinc-inducible $p19^{ARF}$ pMTArf cells (left panel) and doxorubicin (Doxo) -treated wild-type and p53-null ES cells (right panel) were performed as described (10). A rabbit polyclonal antibody to p53 (FL-393) or control rabbit IgG was used. PCR analysis using Dapk1 primers 5′-TACCAGGAAGGGGTGACGGAA-3′, 5′CTGTGCACAGTAACTGCACTGT-3′ and Mdm2 primers 5′GGTGCCTGG TCCCGGACTCGCCGGG-3′ and 5′-CCGAGAGGGTCCCCCAGGGGTGTCC-3′ is shown using input DNA ($\frac{1}{20}$ of ChIP) or DNA after ChIP

(Figure 3b). We next generated luciferase reporter constructs using the genomic regions of both mouse and human Dapk1 that contain the p53-binding elements. These vectors were transiently transfected in Soas2 cells along with a pCMV vector as a control, or with a wild-type p53 expression vector (pCMV-p53). We could conclude from these experiments that both mouse and human p53DNA-binding sites are transcriptionally regulated by p53 (data not shown). Finally, we tested the ability of p53 to bind the mouse p53RE2 *in vivo*, using chromatin immunoprecipitation (ChIp). pMTArf cells were grown for 24 h in the presence of zinc to induce $p19^{ARF}$ expression and subsequent p53 stabilization. Chromatin was immunoprecipitated using either control IgG or p53-specific polyclonal antibodies (FL-393), and the *dapk1* intron 1 region surrounding p53RE2

amplified by PCR (Figure 3c, left panels). Amplification of a region containing the p53-binding sites in the first intron of the *Mdm2* gene served as positive control. Similar experiments were carried out in doxorubicin-treated ES cells (Figure 3c, right panels). Results revealed a recruitment of p53 to the *dapk1* intron 1, thereby proving that *dapk1* is a direct p53-transcription target.

Dapk1 expression is upregulated by hyperproliferative signals such as c-Myc or E2F overexpression (Raveh *et al*., 2001). Since other p53 targets, including Apaf-1, are also transcription targets of c-Myc or E2F1, the expression of *dapk1* may be directly regulated by these oncogenes. However, hyperproliferative signals also lead to the activation of an intrinsic p19^{ARF}-p53-dependent apoptotic checkpoint. We therefore tested whether induction of *dapk1* expression in cells expressing high levels of Myc is dependent on the presence of p53. A tamoxifen-inducible allele of c-Myc (Myc-ER; Zindy *et al*., 1998) was ectopically expressed by retroviral infection in early-passage (p3) wild-type and p53-null MEFs and *dapk1* expression was monitored by semiquantitative PCR (Figure 4). c-Myc expression was comparable in wild-type and p53-null cells, as determined by direct immunoblotting (not shown). As reported, p19^{ARF} expression was significantly elevated following c-Myc activation in wild-type cells, while its basal levels were higher in p53-null fibroblasts (Figure 4; Zindy *et al*., 1998). Importantly, in contrast to wild-type cells, Myc activation did not induce dapk1 transcription levels in the p53-null fibroblasts. Thus, our data demonstrate that p53 is required for oncogene-mediated increases of Dapk1 levels.

Dapk1 has been shown to function upstream of p19^{ARF} and p53 to induce apoptosis through a yet unknown mechanism (Raveh *et al*., 2001). Ectopic expression of Dapk1 suppressed oncogenic transformation of primary fibroblasts by activating p53, in a p19^{ARF}-dependent manner. Furthermore, functional or genetic inactivation of Dapk1 reduced the extent of p53 induction and weakened the subsequent apoptotic responses to oncogene overexpression such as c-Myc or E2F-1 (Raveh *et al*., 2001). Together, these observations and our present data suggest the existence of a positive feedback loop involving Dapk1 and p53, where activation of p53 induces Dapk1 expression, which in turn stimulates p53 stabilization and function. Given that Dapk1 also possesses p53-independent apoptogenic activities (Raveh *et al*., 2001), once activated, this positive feedback loop may contribute to creating a cellular environment that dictates the preferential induction of apoptosis rather than cell-cycle arrest after p53 activation. However, since we observed upregulation of Dapk1 expression even in nonapoptotic tissues in the *mdm4*-null embryos, apoptosis is probably a default program. In specific cellular contexts, mechanisms that disrupt the p53-Dapk1-p19^{ARF}-p53-positive feedback loop may take place to suppress apoptosis. Survival factors might for instance be implicated in the activation of such protective mechanisms. Finally, disruption of this control loop is expected to affect the p53-apoptotic function and therefore have a dramatic impact on its tumour suppressor activity. In agreement with this view, *dapk1* expression is frequently downregulated in human cancers through multiple mechanisms including promoter methylation (Kissil *et al*., 1997; Katzenellenbogen *et al*., 1999; Esteller *et al*., 1999; Sanchez-Cespedes *et al*., 2000). It would be interesting to determine whether inactivating mutations in the *dapk1* and *p53* genes tend to be mutually exclusive during tumorigenesis. Our findings predict that early mutational events in *dapk1* would significantly reduce the pressure for subsequent mutational inactivation of p53.

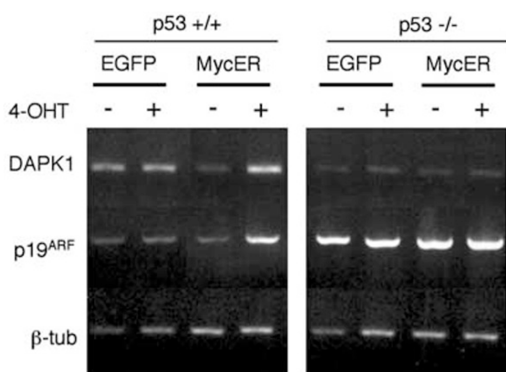

Figure 4 Myc-induced expression of dapk1 is p53 dependent. Myc-ER was introduced in early-passage wild-type MEFs and p53$^{-/-}$ using a retroviral approach, as described (21). Following transduction and selection, 4-hydroxytamoxifen (4-OHT) was added to the MEFs medium at a concentration of 0.6 μM for 24 h. Semiquantitative PCR analysis shows the expression of *dapk1* and p19^{ARF} in p53 wild-type and p53-null MEFs infected with retroviruses coding either for EGFP or tamoxifen-inducible Myc (Myc-ER). Clear induction of dapk1 and p19^{ARF} was observed following treatment only in wild-type cells expressing Myc-ER. β-tub served here as a normalization control

Acknowledgements
We thank Alberto Gobbi and Manuella Capillo for help with mice, Simone Minardi, Loris Bernard and Laura Tizzoni for technical help with the Affymetrix and Q-PCR technologies. We also thank M Rouselle and C Sherr (St Jude Children's Research Hospital, Memphis, TN, USA) for the pMTArf cell line, R Jaenisch (Whitehead Institute for Biomedical Research, Cambridge, USA) for the p53-deficient ES cells and K Vousden for the SAOS-2-p53 cell line. A Martoriati and G Doumont were supported by grants from the FNRS, FRIA and Télévie, respectively. This work was supported in part by grants from FB Insurance, AICR and 'Fédération Belge contre le Cancer'.

References

Alcalay M, Meani N, Gelmetti V, Fantozzi A, Fagioli M, Orleth A, Riganelli D, Sebastiani C, Cappelli E, Casciari C, Sciurpi MT, Mariano AR, Minardi SP, Luzi L, Muller H, Di Fiore PP, Frosina G and Pelicci PG. (2003). *J. Clin. Invest.*, **112,** 1751–1762.

Barak Y, Juven T, Haffner R and Oren M. (1993). *EMBO J.*, **12,** 461–468.

El-Deiry WS. (1998). *Semin. Cancer Biol.*, **8,** 345–357.

Esteller M, Sanchez-Cespedes M, Rosell R, Sidransky D, Baylin SB and Herman JG. (1999). *Cancer Res.*, **59,** 67–70 (Erratum in: *Cancer Res.*, **59,** 3853).

Fiscella M, Zhang H, Fan S, Sakaguchi K, Shen S, Mercer WE, Vande Woude GF, O'Connor PM and Appella E. (1997). *Proc. Natl. Acad. Sci. USA*, **94,** 6048–6053.

Haupt Y, Maya R, Kazaz A and Oren M. (1997). *Nature*, **387,** 296–299.

Hoh J, Jin S, Parrado T, Edington J, Levine AJ and Ott J. (2002). *Proc. Natl. Acad. Sci. USA*, **99,** 8467–8472.

Jeffers JR, Parganas E, Lee Y, Yang C, Wang J, Brennan J, MacLean KH, Han J, Chittenden T, Ihle JN, McKinnon PJ, Cleveland JL and Zambetti GP. (2003). *Cancer Cell*, **4,** 321–328.

Katzenellenbogen RA, Baylin SB and Herman JG. (1999). *Blood*, **93,** 4347–4353.

Kissil JL, Feinstein E, Cohen O, Jones PA, Tsai YC, Knowles MA, Eydmann ME and Kimchi A. (1997). *Oncogene*, **15,** 403–407.

Kuo ML, Duncavage EJ, Mathew R, den Besten W, Pei D, Naeve D, Yamamoto T, Cheng C, Sherr CJ and Roussel MF. (2003). *Cancer Res.*, **63,** 1046–1053.

Lane DP. (1992). *Nature*, **358,** 15–16.

Leng RP, Lin Y, Ma W, Wu H, Lemmers B, Chung S, Parant JM, Lozano G, Hakem R and Benchimol S. (2003). *Cell*, **112,** 779–791.

Levine AJ. (1997). *Cell*, **88,** 323–331.

Marine JC and Jochemsen A. (2004). *Cell Cycle*, **3,** 900–904.

Migliorini D, Denchi EL, Danovi D, Jochemsen A, Capillo M, Gobbi A, Helin K, Pelicci PG and Marine JC. (2002). *Mol. Cell. Biol.*, **22,** 5527–5538.

Nakano K and Vousden KH. (2001). *Mol. Cell*, **7,** 683–694.

Okamura S, Arakawa H, Tanaka T, Nakanishi H, Ng CC, Taya Y, Monden M and Nakamura Y. (2001). *Mol. Cell*, **8,** 85–94.

Raveh T, Droguett G, Horwitz MS, DePinho RA and Kimchi A. (2001). *Nat. Cell Biol.*, **3,** 1–7.

Sanchez-Cespedes M, Esteller M, Wu L, Nawroz-Danish H, Yoo GH, Koch WM, Jen J, Herman JG and Sidransky D. (2000). *Cancer Res.*, **60,** 892–895.

Schmitt CA, Fridman JS, Yang M, Baranov E, Hoffman RM and Lowe SW. (2002). *Cancer Cell*, **1,** 289–298.

Takekawa M, Adachi M, Nakahata A, Nakayama I, Itoh F, Tsukuda H, Taya Y and Imai K. (2000). *EMBO J.*, **19,** 6517–6526.

Villunger A, Michalak EM, Coultas L, Mullauer F, Bock G, Ausserlechner MJ, Adams JM and Strasser A. (2003). *Science*, **302,** 1036–1038.

Vousden KH and Lu X. (2002). *Nat. Rev. Cancer*, **2,** 594–604.

Zindy F, Eischen CM, Randle DH, Kamijo T, Cleveland JL, Sherr CJ and Roussel MF. (1998). *Genes Dev.*, **12,** 2424–2433.

Supplementary information accompanies the paper on http://www.dmbr.ugent.be/lmcb/contents/p53targets/.