Oncogene (1999) 18, 7740–7747

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p53 induces TAP1 and enhances the transport of MHC class I peptides

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The transporter associated with antigen processing (TAP) 1 is required for the major histocompatibility complex (MHC) class I antigen presentation pathway, which plays a key role in host tumor surveillance. Since more than 50% of tumors have a dysfunctional p53, evasion of tumor surveillance by tumor cells may be linked to loss of p53 function. Here we found that TAP1 is strongly induced by p53 and DNA-damaging agents through a p53-responsive element. We also found that p73, which is homologous to p53, is capable of inducing TAP1 and cooperates with p53 to activate TAP1. Furthermore, we found that by inducing TAP1, p53 enhances the transport of MHC class I peptides and expression of surface MHC-peptide complexes, and cooperates with interferon γ to activate the MHC class I pathway. These results suggest that tumor surveillance may be a mechanism by which p53 and/or p73 function as tumor suppressors.

Keywords: p53; TAP1; MHC class I; interferon γ; tumor surveillance

Introduction

p53 is one of the most frequently mutated genes in cancer. More than 50% of all human tumors contain a dysfunctional p53 (Hollstein *et al*., 1991). It is well established that p53 plays an important role in the regulation of cell cycle, apoptosis, differentiation, and in the maintenance of genome integrity (Chen, 1999; Almog and Rotter, 1998; Ko and Prives, 1996; Levine, 1997), all of which contribute to p53 tumor suppression. As a sequence-specific transcription factor, p53 up-regulates expression of several cellular genes, for example, p21 and 14-3-3σ that mediate p53-dependent cell cycle arrest (el-Deiry *et al*., 1993; Hermeking, 1997), and BAX and a group of redox-related genes (PIGs) that may mediate p53-dependent apoptosis (Miyashita *et al*., 1994; Polyak *et al*., 1997).

p53 is a multifunctional protein. Mechanisms other than cell cycle arrest and apoptosis may also be involved in p53 tumor suppression. When normal cells become malignant, cellular proteins that are normally present at low levels may become over-expressed or the genes that encode these cellular proteins may become mutated, resulting in the production of tumor antigens (Old and Chen, 1998). These tumor antigens would then be processed and presented by the host major histocompatibility complex (MHC) class I antigen presentation pathway on the cell surface. Several proteins are necessary for the MHC class I pathway, including large multifunctional proteasome subunits 2 and 7 (LMP2 and LMP7), transporters associated with antigen processing 1 and 2 (TAP1 and TAP2), and two polypeptides for the MHC class I molecule, heavy chain HLA-ABC and light chain β_2 microglobulin (β_2M) (Pamer and Cresswell, 1998). LMP2 and LMP7 are involved in breaking down intracellular proteins into antigenic peptides. TAP1 and TAP2 are involved in the transport of these antigenic peptides from cytosol to endoplasmic reticulum where they bind to the assembled MHC class I molecules. The MHC-peptide complex is then transported to and expressed on the cell surface. Cytotoxic T lymphocytes (CTLs) recognize and attack cells with tumor antigens on the cell surface via an interaction between the T cell receptor and the MHC-peptide complex. However, during tumorigenesis, tumor cells acquire mutations that help them evade recognition by the immune system. One mechanism that tumor cells could use is to down-regulate the MHC class I pathway (Pamer and Cresswell, 1998; Restifo *et al*., 1993b). Without stable MHC-peptide complexes on the cell surfaces, tumor cells evade CTL recognition.

As part of our ongoing effort to understand p53 function in cells, we used the ClonTech PCR-Select cDNA Subtraction assay to identify novel cellular p53 target genes. We found that TAP1 is specifically induced by both p53 and p73, which leads to enhanced transport of MHC class I peptides. These findings suggest that tumor surveillance can be mediated by the p53 family tumor suppressor proteins.

Results

Upregulation of TAP1 by p53

In an effort to identify new p53 target genes, the ClonTech PCR-Select cDNA Subtraction assay was performed using mRNA isolated from p53-3, a derivative of H1299 cell line that inducibly expresses p53 under a tetracycline-regulated promoter (Chen *et al*., 1996b). Several cDNA fragments that may represent genes activated by p53 were isolated. After DNA sequencing, one subtracted cDNA fragment was found to be derived from the TAP1 gene. To confirm that TAP1 can be induced by p53, Northern blot analysis was performed using TAP1 cDNA as probe. We found that TAP1 was induced in p53-3 cells when p53 was expressed (Figure 1a, compare lanes 1 and 2). As a control, we tested expression of p21, a well-defined cellular p53 target gene (el-Deiry *et al*., 1993).

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Received 24 May 1999; revised 9 September 1999; accepted 14 September 1999

We found that p21 was also induced by p53 (Figure 1a, compare lanes 1 and 2). Furthermore, we found that mutant p53(R249S) was incapable of activating both TAP1 and p21 (Figure 1a, compare lanes 3 and 4), consistent with the fact that this tumor-derived p53 mutant is defective in transactivation. After normalization to the level of GAPDH mRNA, we estimated that the amount of TAP1 in cells expressing p53 was 4–6 times higher than in cells not expressing p53.

Since the p53 protein is stabilized and accumulates in cells following DNA damage (Ko and Prives, 1996), we determined whether TAP1 can be activated by DNA damage in the RKO colorectal carcinoma cell line, which contains an endogenous wild-type p53 gene (Nelson and Kastan, 1994). To this end, RKO cells were treated with camptothecin, doxorubicin, or actinomycin D. Camptothecin and doxorubicin are inhibitors of topoisomerase I and II, respectively, both of which induce double-strand DNA breaks (Nelson and Kastan, 1994). Actinomycin D inhibits transcription, but induces DNA damage at low concentrations (1–10 nM) (Nelson and Kastan, 1994). Northern blot analysis showed that TAP1 was induced in RKO cells treated with these DNA-damaging agents (Figure 1b). As expected, p21 was also activated (Figure 1b). After normalization to the level of GAPDH mRNA, we found that the amount of TAP1 expressed in RKO cells treated with these DNA-damaging agents was 4–8 times greater than in mock-treated cells.

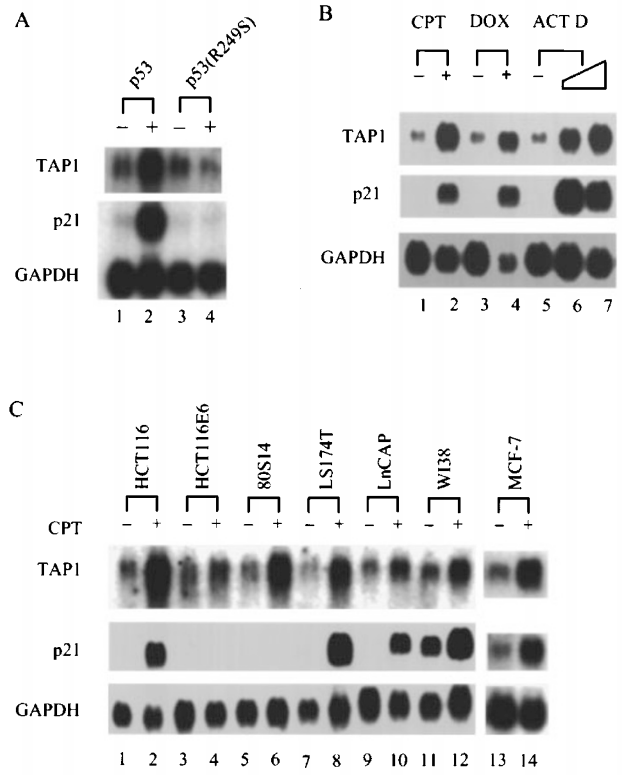

Figure 1 (**a**) Wild-type p53, but not p53 mutant, induces TAP1. A Northern blot was prepared using 10 μg of total RNA isolated from p53-3 or p53(R249S)-2 cells that were uninduced (−) or induced (+) to express wild-type p53 and mutant p53(R249S), respectively. (**b**) TAP1 is induced by three DNA-damaging agents in RKO cells. A Northern blot was prepared using 10 μg of total RNA isolated from untreated RKO cells (−) or cells treated (+) with 300 nM camptothecin (CPT), 1.0 μg/ml doxorubicin (DOX), 3.0 or 10 nM actinomycin D (ACT D). (**c**) TAP1 is induced by DNA damage in six cell lines that carry an endogenous wild-type p53 gene but not in one that is functionally p53-null. Northern blots were prepared using 10 μg of total RNA isolated from seven individual cell lines as indicated at the top of the figure, which were untreated (−) or treated (+) with 300 nM camptothecin for 24 h. The blots were probed with TAP1 cDNA, and then reprobed with p21 and GAPDH cDNAs, respectively

If TAP1 is a true cellular p53 target, TAP1 should be induced by p53 (i.e., DNA damage) in other cell lines that contain an endogenous wild-type p53 gene but not in cell lines that are p53-null. To this end, we tested seven different cell lines. HCT116, LS174T, LnCap, WI-38, and MCF7 each contain an endogenous wild-type p53 gene. 80S14 cell line is an HCT116 derivative that is p21-null (Waldman *et al*., 1996), and HCT116E6 is an HCT116 derivative that contains human papillomavirus (HPV) oncoprotein E6. Since HPV E6 facilitates degradation of p53 (Ko and Prives, 1996), HCT116E6 is a p53-null-like cell line. These cells were treated with camptothecin and the levels of TAP1 and p21 determined by Northern blot analysis (Figure 1c). We found that both TAP1 and p21 were induced in cells containing wild-type p53 when treated with camptothecin (Figure 1c, lanes 1,2 and 7–14). Although p21 was not expressed in the p21-null 80S14 cells, TAP1 was still induced by DNA damage (Figure 1c, lanes 5 and 6), indicating that p53 can activate TAP1 independently of p21. In contrast, TAP1 was not induced in p53-null-like HCT116E6 cells (Figure 1c, lanes 3 and 4).

Since p73 is homologous to p53 (Kaghad *et al*., 1997) and is capable of inducing p21 (Jost *et al*., 1997; Kaghad *et al*., 1997; Zhu *et al*., 1998a), we wanted to determine whether TAP1 is a common cellular target of p53 and p73. To this end, we used three H1299 cell lines that inducibly express two alternatively spliced forms of wild-type p73, i.e., p73α and p73β, and one mutant p73α292, respectively (Zhu *et al*., 1998a). We found that both TAP1 and p21 were induced by both wild-type p73α and p73β but not by mutant p73α292 (Figure 2a). Since both p53 and p73 are activators of transcription, they may cooperate to activate genes responsible for tumor suppression. To determine whether TAP1 is activated cooperatively by p53 and p73, TAP1 expression was examined in MCF7 cells that are either induced to express p73α, treated with camptothecin to induce p53, or both induced to express p73α and treated with camptothecin to induce p53 (Figure 2b). We found that TAP1 was up-regulated in MCF7 cells when treated with camptothecin (Figure 2b, compare lanes 1 and 2) or induced to express p73α (Figure 2b, compare lanes 1 and 4). After PhosphorImage quantitation, we found that TAP1 was induced 2.6-fold by either p53 or p73α. In contrast, TAP1 was induced 7.1-fold when both p53 and p73α were expressed in MCF7 cells (Figure 2b, lane 3). These results suggest that p73α and p53 (DNA damage) cooperate to activate TAP1 expression. We also found that p21 was activated cooperatively by p73α and DNA damage-induced p53 in MCF7 cells (Figure 2b).

Because TAP1 is one of the components required for the MHC class I antigen presentation pathway, we wanted to determine whether other genes in this pathway are regulated by p53. We examined five other genes by Northern blot analysis and found that TAP2, LMP2, LMP7 and MHC class I heavy chain

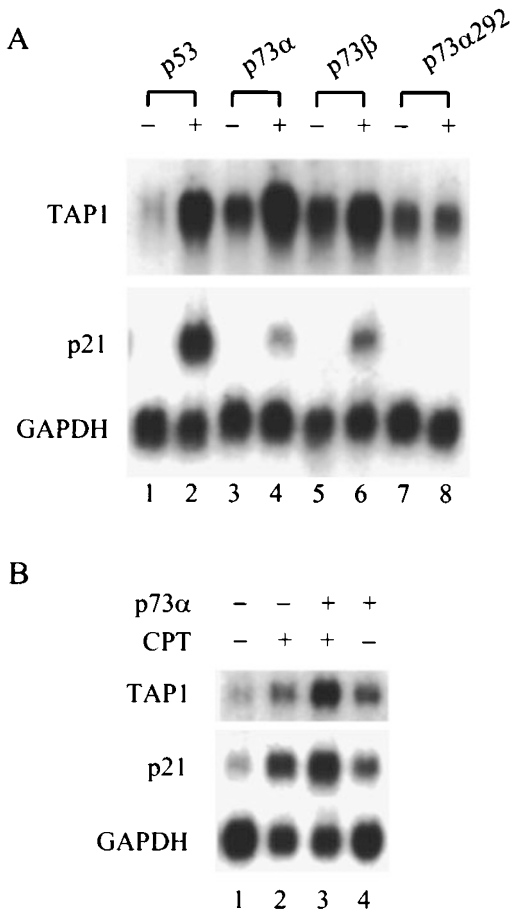

Figure 2 (**a**) Wild-type p73, but not mutant p73, is capable of inducing TAP1. A Northern blot was prepared using 10 μg of total RNA isolated from uninduced cells (lanes 1, 3, 5 and 7) or cells that were induced to express wild-type p53 (lane 2), p73α (lane 4), p73β (lane 6), or mutant p73α292 (lane 8). (**b**) p73 cooperates with DNA damage to activate TAP1 in MCF7 cells that carry an endogenous wild-type p53 gene. A Northern blot was prepared using 10 μg of total RNA isolated from MCF7 cells that were untreated (lane 1), treated with 300 nM camptothecin (CPT) to induce endogenous wild-type p53 (lane 2), induced to express exogenous p73α and treated with 300 nM camptothecin to induce endogenous wild-type p53 (lane 3), or induced to express exogenous p73α (lane 4). The blots were probed with TAP1, p21, and GAPDH cDNAs, respectively

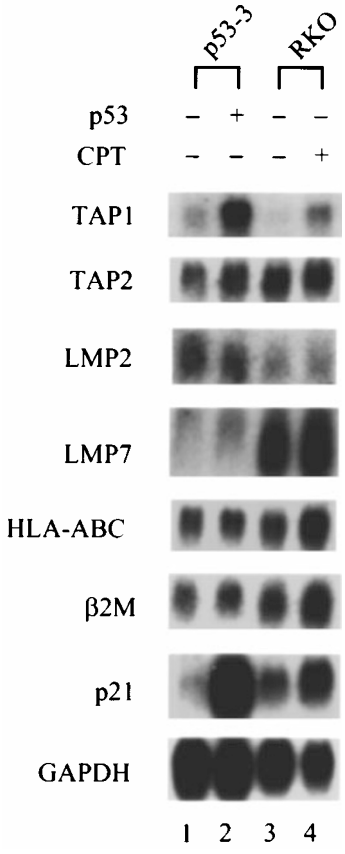

Figure 3 TAP1, but not other components in the MHC class I pathway, is induced by p53. Northern blots were prepared using 10 μg of total RNA isolated from p53-3 cells that were uninduced (−) or induced (+) to express exogenous wild-type p53, or from RKO cells that were untreated (−) or treated (+) with 300 nM camptothecin to induce endogenous wild-type p53 for 24 h. The blots were probed with TAP1, TAP2, LMP2, LMP7, HLA-ABC, β_2M, p21 and GAPDH cDNAs, respectively

HLA-ABC and light chain β_2M were expressed, but not significantly induced by p53 or DNA damage in p53-3 and RKO cells, respectively (Figure 3).

Next, we examined the level of TAP1 protein in p53-3 cells by Western blot analysis. We found that p53 expression resulted in the increase of TAP1 protein (Figure 4, compare lanes 1 and 2), consistent with p53 induction of TAP1 mRNA as analysed by Northern blot analysis (Figure 1a). p53-3 cells were also treated with 5, 15, 50, 100 and 500 U of IFNγ, a potent inducer of TAP1 (Stark *et al*., 1998). We found that the TAP1 protein was efficiently induced with 15 U/ml of IFNγ (Figure 4, lane 3).

Identification of a specific p53-responsive element in the TAP1 gene

To define whether TAP1 is a true target of p53, we searched for a p53-responsive element in the genomic DNA sequence of the TAP1 gene. A potential p53-

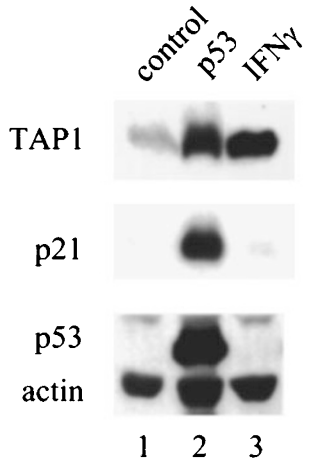

Figure 4 The TAP1 protein is increased in cells expressing p53 or treated with IFNγ. The levels of TAP1, p21, p53, and actin proteins in p53-3 cells that were untreated (lane 1), induced to express p53 (lane 2), or treated with 15 U/ml of IFNγ (lane 3), were assayed by Western blot analysis. The blots were probed with anti-TAP1 monoclonal antibody A148.3, anti-p21 monoclonal antibody, and a mixture of anti-p53 monoclonal antibody Pab1801 and anti-actin polyclonal antibody, respectively

binding site was found to be located approximately 300 nucleotides downstream of the TAP1 transcription start site (Beck *et al*., 1992). This sequence (ggg cttg g*cc ctgccg gga cttg cct) has only one mismatch (G* instead of C/T) to the consensus p53-binding site (el-Deiry *et al*., 1992). To analyse whether p53 binds to this sequence, a 59-bp DNA fragment containing this region was synthesized, ^{32}P-labeled, and used in an electrophoretic mobility shift assay (EMSA). We found that p53 interacts specifically with the potential p53-responsive element in the TAP1 gene (data not shown).

We further examined whether the potential p53-binding site is responsive to p53 *in vivo*. To do this, the potential p53-responsive element was cloned upstream of a minimal promoter and a luciferase reporter gene to generate the reporter vector TAP1-Fluc. The construct GADD45-Fluc, which contains a p53-responsive element from the GADD45 gene, a well-defined cellular p53 target, was used as a positive control as described previously (Chen *et al*., 1995). We found that the luciferase activity for either TAP1-Fluc or GADD45-Fluc was markedly increased by wild-type p53 (Figure 5a), suggesting that p53 can bind to the p53-responsive elements from both the TAP1 and GADD45 genes. Interestingly, we observed that the increase in the luciferase activity by p53 for TAP1-Fluc was about five times greater than that for GADD45-Fluc (Figure 5a). This suggests that the p53-binding site in the TAP1 gene may have a higher affinity for p53 than the binding site in the GADD45 gene. Similarly, we found that the luciferase activity for TAP1-Fluc was increased by both p73α and p73β (Figure 5b). In contrast, the luciferase activity for TAP1-Fluc was not increased by the mutants p53(V143A), p53(R175H), p53(R249S), or p53(R273H) (Figure 5c), consistent with the observation that mutant p53(R249S) was incapable of inducing TAP1 (Figure 1a).

p53 induction of TAP1 leads to increased transport of MHC class I peptides

To determine whether induction of TAP1 by p53 can lead to increased transport of MHC class I peptides,

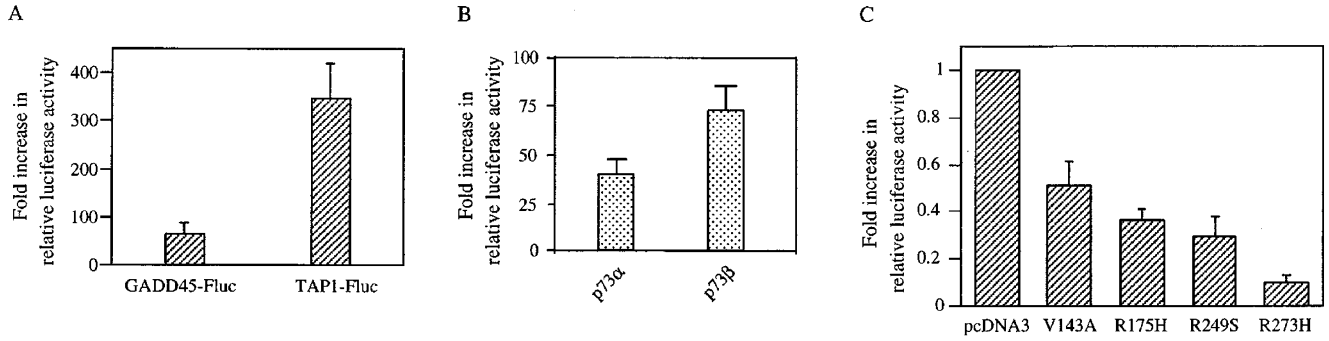

Figure 5 Wild-type p53 and p73 bind to the p53-responsive element *in vivo*. (**a**) The potential p53-binding site in the TAP1 gene is responsive to wild-type p53 *in vivo*. 5 μg of TAP1-Fluc or GADD45-Fluc was co-transfected into H1299 cells with 5 μg of pcDNA3 or a vector that expresses wild-type p53. The fold increase in relative luciferase activity is a product of the luciferase activity activated by p53 divided by that activated by pcDNA3. (**b**) The potential p53-binding site in the TAP1 gene is responsive to wild-type p73 *in vivo*. 5 μg of TAP1-Fluc was co-transfected into H1299 cells with 5 μg of pcDNA3 or a vector that expresses wild-type p73α or p73β. The fold increase in relative luciferase activity is calculated similarly to that in (**a**). (**c**) p53 mutants are unable to increase the luciferase activity for TAP1-Fluc. 5 μg of TAP1-Fluc was co-transfected into H1299 cells with 5 μg of pcDNA3 or a vector that expresses p53(V143A), p53(R175H), p53(R249S), or p53(R273H). The fold increase in relative luciferase activity was determined similarly to that in (**a**)

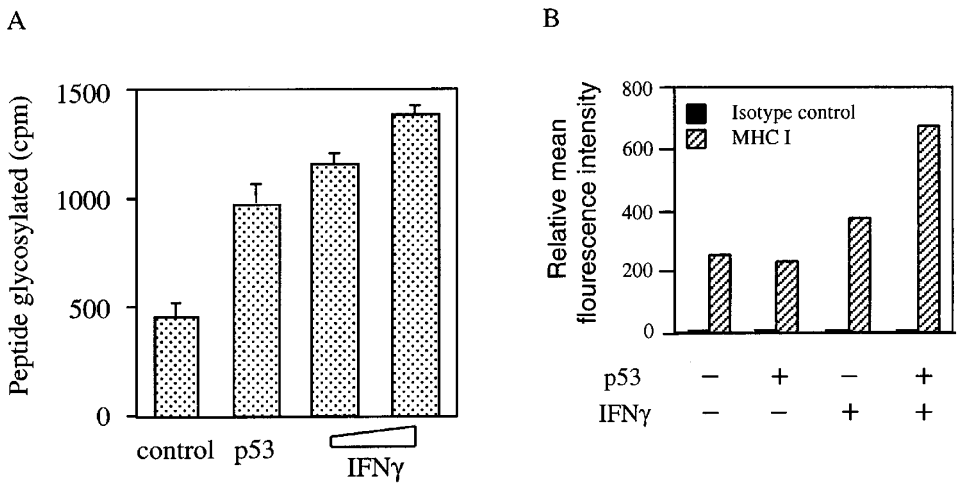

Figure 6 (**a**) p53 and IFNγ increases peptide transport capacity in p53-3 cells. p53-3 cells were uninduced, induced to express p53, or treated with 5 or 20 U/ml of IFNγ for 24 h. The extent of peptide glycosylation was then used to measure the relative peptide transport capacity in cells. (**b**) p53 cooperates with IFNγ to enhance the expression of surface MHC-peptide complexes. p53-3 cells that were uninduced or induced to express p53 were mock-treated or treated with 500 U/ml IFNγ for 48 h. The level of surface MHC-peptide complexes was determined by FACS analysis with anti-human HLA-ABC antibody B-H9. Mouse IgG1 was used as an isotype control

we performed peptide transport assays (Ma *et al*., 1997). We found that the amount of glycosylated B27 peptide, a variant of an HLA-B27-binding, human histone 3 peptide, was significantly increased in p53-3 cells by p53 and IFNγ (Figure 6a). Similar results were obtained with A3 peptide, a variant of an HLA-A3-binding, HIV nef 7B peptide (data not shown). It should be noted that since IFNγ can also induce TAP2, the other key component for the transport of MHC class I peptides (Pamer and Cresswell, 1998), it is not surprising that IFNγ was more potent than p53 in enhancing the transport of B27 peptide (Figure 6a).

As MHC class I peptides are transported into the endoplasmic reticulum, they bind to assembled MHC class I molecules to form stable MHC-peptide complexes, which are subsequently expressed on the cell surface (Pamer and Cresswell, 1998). To determine whether p53 can increase the expression of surface MHC-peptide complexes on p53-3 cells, FACS analysis was performed. We found that the level of surface MHC-peptide complexes was not significantly increased by p53 (Figure 6b). This is not surprising since other abnormalities in the MHC class I pathway can inhibit MHC class I expression (Proffitt and Blair, 1997; Restifo *et al*., 1993a). Indeed, the LMP7 gene, whose product is required for the generation of MHC class I peptides, was found to be expressed at an extremely low level in p53-3 cells (Figure 3). Consequently, the supply of cellular MHC class I peptides may be limited, which hinders the formation of stable MHC-peptide complexes. Therefore, we examined whether p53 can further increase MHC class I expression when p53-3 cells are treated with IFNγ to induce LMP7. We found that the level of MHC-peptides complexes expressed on IFNγ-treated cells was about 1.5 times higher than on untreated cells or cells expressing p53 (Figure 6b). However, when cells were both induced to express p53 and treated with IFNγ, the level of surface MHC-peptide complexes was 2.6 times greater than on untreated cells or cells expressing p53 alone (Figure 6b).

Since the LMP7 gene is highly expressed in the RKO cell line (Figure 3, LMP7 panel), we chose it to further determine whether p53 can enhance the transport of MHC class I peptides and expression of surface MHC-peptide complexes. As expected, when RKO cells were treated with camptothecin, the p53 protein was stabilized (Figure 7a, p53 panel), and subsequently, the TAP1 mRNA (Figure 7b, TAP1 panel) and protein (Figure 7a, TAP1 panel) upregulated. When RKO cells were treated with IFNγ, p53 was not stabilized (Figure 7a, p53 panel), but the TAP1 mRNA (Figure 7b, TAP1 panel) and protein (Figure 7a, TAP1 panel) were increased, suggesting that IFNγ can regulate the MHC class I pathway independently of p53 in RKO cells. To determine whether p53 is necessary for the enhanced expression of TAP1, we generated a derivative of the RKO cell line, RE6-26, which stably expresses HPV E6 oncoprotein. As a result, RE6-26 becomes a p53-null-like cell line. Indeed, p53 was undetectable in RE6-26 cells when treated with camptothecin (Figure 7a, compare lanes 4 and 5, p53 panel) and subsequently, the TAP1 mRNA (Figure 7b, TAP1 panel) and protein (Figure 7a, TAP1 panel) not induced. However, TAP1 was still induced in RE6-26 cells by IFNγ (Figure 7a, TAP1 panel; Figure 7b, TAP1 panel), suggesting that the IFNγ-regulated MHC class I pathway is not affected by the HPV E6 oncoprotein.

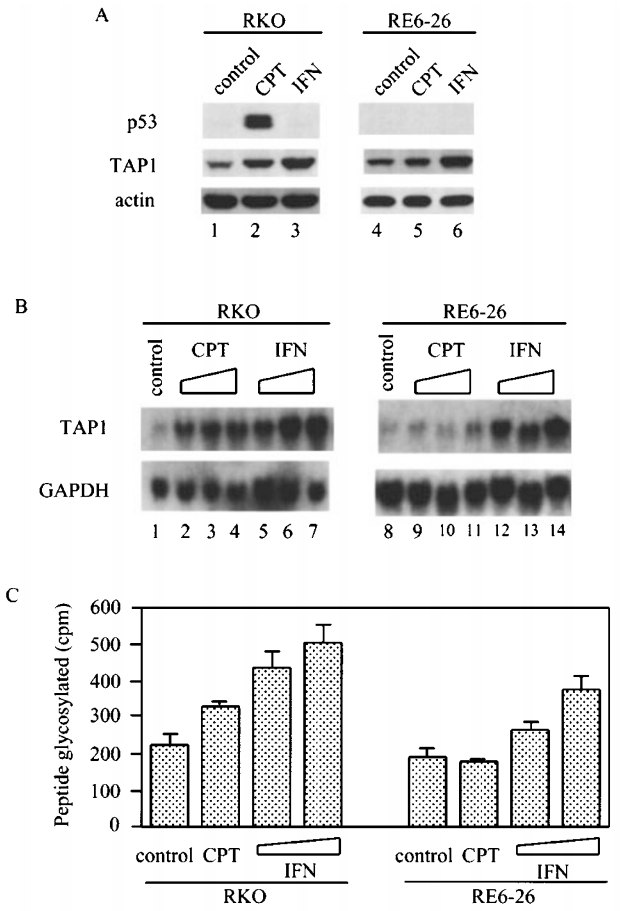

Figure 7 (**a**) p53 is required for the increased expression of TAP1 protein in RKO cells by DNA damage. The levels of TAP1, p53, and actin proteins in RKO and RE6-26 cells that were untreated (lanes 1 and 4), treated with 100 nM camptothecin (lanes 2 and 5), or 20 U/ml IFNγ (lanes 3 and 6), were assayed by Western blot analysis. The blots were probed with anti-p53 Pab1801, anti-TAP1 A148.3, and anti-actin polyclonal antibody, respectively. (**b**) p53 is required for the increased expression of TAP1 mRNA in RKO cells by DNA damage. Northern blots were prepared using 10 μg of total RNA isolated from RKO or RE6-26 cells that were untreated (lanes 1 and 8), treated with 50, 100, or 200 nM camptothecin (lanes 2–4 and 9–11), or treated with 10, 20, or 40 U/ml IFNγ (lanes 5–7 and 12–14) for 24 h. The blots were probed with TAP1 cDNA, and then reprobed with GAPDH cDNA. (**c**) p53 is required for the increased peptide transport capacity in RKO cells by DNA damage. Peptide transport assay was performed using RKO or RE6-26 cells that were untreated, treated with 100 nM camptothecin, or treated with 5 or 20 U/ml IFNγ for 24 h. The extent of peptide glycosylation was then used to measure the relative peptide transport capacity in cells

Next, we determined the peptide transport capacity in RKO and RE6-26 cells when treated with camptothecin or IFNγ. We found that the amount of glycosylated peptide was increased in RKO cells by both camptothecin and IFNγ (Figure 7c). In contrast, IFNγ, but not camptothecin, was capable of increasing the transport of MHC class I peptides in RE6-26 cells (Figure 7c).

To determine whether DNA damage can increase the expression of surface MHC-peptide complexes, RKO cells were treated with 0, 10, 25, 50, 250 and

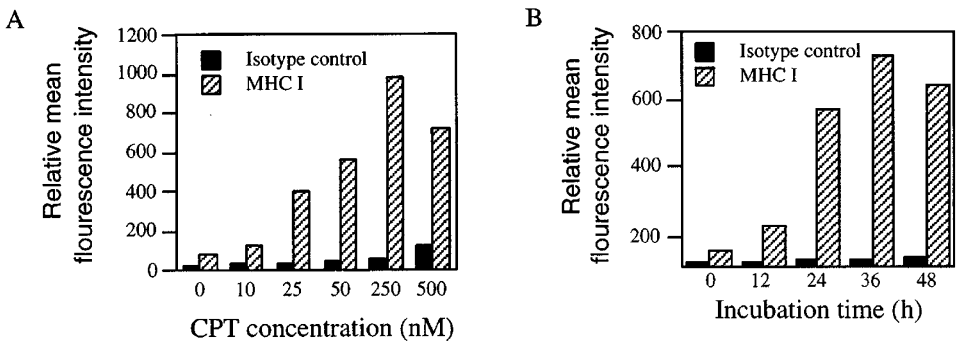

Figure 8 DNA damage increases the expression of surface MHC-peptide complexes on RKO cells when treated with camptothecin in dose- and time-dependent manners. (**a**) RKO cells were treated with 0, 10, 25, 50, 250 and 500 nM camptothecin for 24 h. (**b**) RKO cells were treated with 250 nM camptothecin for 0, 12, 24, 36 and 48 h. The level of surface MHC-peptide complexes was determined with anti-human HLA-ABC antibody B-H9. Mouse IgG1 was used as an isotype control

500 nM camptothecin for 24 h or treated with 250 nM camptothecin for 0, 12, 24, 36 and 48 h. We found that the level of surface MHC-peptide complexes was increased markedly in RKO cells by DNA damage in dose- and time-dependent manners (Figure 8a, b). In contrast, DNA damage had no effect on the MHC class I expression in RE6-26 cells (data not shown). These results suggest that p53 is responsible for the upregulation of the MHC class I pathway following DNA damage.

Discussion

In this study we have demonstrated that TAP1 can be induced by both p53 and several DNA-damaging agents. The induction of TAP1 by DNA damage is p53-dependent because TAP1 is not induced in cells when p53 is functionally null. We found that this induction is mediated by a p53-responsive element located 300 nucleotides downstream of the TAP1 transcription start site. Furthermore, the newly synthesized, p53-induced TAP1 protein is functional in increasing the transport of MHC class I peptides and subsequent expression of surface MHC-peptide complexes.

Since the MHC class I pathway is critical for host tumor surveillance (Pamer and Cresswell, 1998), tumor cells could evade tumor surveillance by acquiring mutations that inhibit the MHC class I pathway. Thus, mutation of one or more of the genes that encode key components for the MHC class I pathway would diminish or abrogate the host tumor surveillance. Indeed, the genes that encode the MHC heavy chain HLA-ABC and light chain β2M were found to be mutated in melanoma tumors (D'Urso *et al*., 1991; Restifo *et al*., 1993a). In adenovirus 12-transformed cells, the expression of the LMP2 gene was inhibited by adenoviral oncoproteins (Deiss and Kimchi, 1991; Proffitt and Blair, 1997). Interestingly, mutations that affect TAP1 occur frequently in a variety of human tumors (Amiot *et al*., 1998; Chen *et al*., 1996a; Cromme *et al*., 1994; Kaklamanis *et al*., 1995; Khanna *et al*., 1998) and tumor cell lines (Alpan *et al*., 1996; Johnsen *et al*., 1998; Restifo *et al*., 1993a; Vitale *et al*., 1998; Wang *et al*., 1998). Here we found that the tumor suppressor p53 is necessary for inducing TAP1 in cells following DNA damage. Thus, a dysfunctional p53 in more than 50% of human tumor cells would not induce TAP1 following genotoxic stress.

How does this novel activity of p53 relate to the central role of p53 in tumor suppression? p53 is a well-defined checkpoint protein in the cell cycle (Almog and Rotter, 1998; Ko and Prives, 1996; Levine, 1997). When cells are exposed to extracellular or intracellular stresses, for example, DNA damage, p53 is stabilized, resulting in cell cycle arrest, apoptosis, or differentiation. Cells suffering from DNA damage often express abnormal cellular proteins that need to be processed and presented on the cell surface (Old and Chen, 1998). These cells are then recognized by the host immune system, leading to their elimination. Our data suggest that p53 also activates the MHC class I pathway by inducing TAP1, which would facilitate this process. If tumor cells acquire additional mutations that inactivate p53, this process of tumor surveillance would be curtailed. Similarly, when oncogenic tumor viruses invade cells, viral proteins are expressed in cells, and then are processed and expressed on the cell surfaces by the MHC class I pathway, leading to elimination of the infected cells (McMichael, 1998; Ploegh, 1998). However, viral oncoproteins, such as HPV E6, adenoviral E1B, and hepatitis B virus (HBV) X, inactivate p53 (Ko and Prives, 1996), which in turn would abrogate the p53-dependent activation of TAP1. We have shown here that HPV E6 oncoprotein does just this in RKO and HCT116 cells. Subsequently, the infected cells would evade recognition by the host immune system and become transformed. Thus, we hypothesize that p53 may have a function in tumor surveillance and inactivation of p53 may be one mechanism that tumor cells use to evade host tumor surveillance.

The MHC class I pathway has been found to be defective in several neuroblastoma cell lines (Cheng *et al*., 1996), which also carry a hemizygous deletion of a 9 cM interval on chromosome 1p35-36.1 where the p73 gene is located (Kaghad *et al*., 1997). Since p73 is expressed from only one allele in some cells due to genomic imprinting (Kaghad *et al*., 1997), a hemizygous deletion of the expressible allele would result in total loss of p73 expression. In this study, we found that p73 is capable of activating the TAP1 gene. Thus,

consistent with the previous observation, loss of p73 may be responsible for down-regulation of the MHC class I pathway in some neuroblastoma cells.

IFN-γ is the most potent inducer of the MHC class I pathway (Stark *et al*., 1998). Upon binding to its receptor, IFNγ activates the Jak/Stat signaling pathway, leading to induction of at least two groups of transcriptional activators, i.e., the IFN regulatory factors (IRFs) and the class II transactivator (CIITA). IRFs bind to the IFN-stimulated response element (ISRE) and activate several genes in the MHC class I pathway, including the TAP1 gene (Pamer and Cresswell, 1998; Stark *et al*., 1998). CIITA binds to the site α in the MHC class I heavy chain genes and activates HLA-ABC expression (Gobin *et al*., 1997; Martin *et al*., 1997). Since the induction of TAP1 by IFNγ occurs in H1299 cells that are p53-null (Figure 4), the regulation of the MHC class I pathway by IFNγ is independent of p53. A recent report showed that IFNγ-insensitive $p53^{-/-}$ mice develop tumors more rapidly with a broader spectrum of tumors when compared to either $p53^{-/-}$ mice or IFNγ-insensitive mice individually (Kaplan *et al*., 1998). Furthermore, we found that p53 can cooperate with IFNγ to activate the MHC class I pathway. Thus, it is likely that tumor cells lacking both p53 and an IFNγ response would be defective in the MHC class I antigen presentation pathway, and such cells would become less immunogenic.

Materials and methods

Cell culture

H1299, HCT116, LS174T, LnCap, MCF-7 and WI-38 cell lines were purchased from American Type Culture Collection. RKO cells were cultured as described (Nelson and Kastan, 1994). 8OS14 cell line was cultured as described (Waldman *et al*., 1996). RE6-26 and HCT116E6 are derivatives of RKO and HCT116, respectively, which were stably transfected with the E6 gene from human papilloma virus (HPV) 16 (Munger *et al*., 1989). p53-3 and p53(R249S)-2 cell lines, derivatives of H1299 that inducibly express wild-type p53 and p53(R249S), respectively, were cultured as described (Chen *et al*., 1996b). The H1299 cell lines that inducibly express p73α, p73α292 and p73β are p73α-22, p73α292-20 and p73β-9, respectively, as previously described (Zhu *et al*., 1998a). The MCF7 cell line, which expresses tet-VP16 for generation of tetracycline inducible cell lines, was purchased from ClonTech (Palo Alto, CA, USA). MCF7 cell lines that express inducible proteins of interest were generated as previously described (Chen *et al*., 1996b). Camptothecin, doxorubicin, and actinomycin D were purchased from Sigma (St. Louis, MC, USA). Human recombinant IFNγ was purchased from Boehringer Mannheim Biochemical (Germany).

RNA isolation, cDNA subtraction assay, and Northern blot analysis

Poly(A)$^+$ RNA was isolated from p53-3 cells using mRNA purification kit (Pharmacia, Piscataway, NJ, USA). Total RNA was isolated from cells using Trizol reagents (Life Technologies, Inc., Gaithersburg, MD, USA). cDNA subtraction assay was performed using ClonTech PCR-Select cDNA Subtraction kit (ClonTech, Palo Alto, CA, USA). Northern blot analysis was performed as described previously (Zhu *et al*., 1998a). p21 and GAPDH probes were prepared as described previously (Zhu *et al*., 1998b). TAP1 probe, a 800-bp *Sma*I – *Hind*III fragment, was prepared from human TAP1 cDNA. LMP2 and TAP2 probes were generated by RT – PCR as described previously (Restifo *et al*., 1993a). HLA-ABC probe was prepared from mouse H2-K^b cDNA. β_2M cDNA probe (GenBank # AA143790) and LMP7 cDNA probe (AA147042) were purchased from Genome System Inc. (St. Louis, MO, USA).

Electrophoretic mobility shift assay (EMSA) and luciferase assay

Purification of the p53 protein and EMSA were performed as described previously (Chen *et al*., 1993). The EMSA probe was a 59-bp fragment containing a potential p53-binding site (underlined) in the TAP1 gene: 5′-atcgacgtaagcttctg-cagggcttgg*ccctgccgsggacttgcctagatctacgt-3′. For luciferase assay, the fragment was cloned upstream of a minimal c-*fos* promoter and a firefly luciferase reporter gene (Johansen and Prywes, 1994), and the resulting construct designated TAP1-Fluc. GADD45-Fluc was described previously (Chen *et al*., 1995). TAP1-Fluc or GADD45-Fluc was co-transfected into H1299 cells with control vector pcDNA3 or a vector that expresses wild-type p53, p53(V143A), p53(R175H), p53(R249S), p53(R273H), p73α or p73β. Dual luciferase assay was performed according to the manufacturer's instructions (Promega).

Western blot analysis

Western blot analysis was performed as described previously (Zhu *et al*., 1998b). Anti-human TAP1 monoclonal antibody, Ab148.3, was kindly provided by Dr B Seliger (Meyer *et al*., 1994). Antibodies against p53, p21, actin were described previously (Zhu *et al*., 1998a).

Peptide, peptide labeling and peptide transport assay

Two MHC class I peptides were synthesized by Molecular Biology Core Facility (Medical College of Georgia) for use in the transport assay. These were: B27, a variant of an HLA-B27-binding, human histone 3 peptide (RRYQ*N*STEL), where Asn is substituted for Lys (Ma *et al*., 1997); and A3, a variant of an HLA-A3-binding, HIV nef 7B peptide (QVPLR*N*MTYK), where Asn is substituted for Pro (Ma *et al*., 1997). The peptides were labeled with Na ^{125}I (Amersham Pharmacia) and purified through a sephadex G-25 column. The specific activity of the labeled peptides was approximately 100 c.p.m./fmol. Transport assay was performed as previously described (Ma *et al*., 1997).

FACS analysis

FACS analysis was performed as previously described (Ma *et al*., 1997). FITC-labeled mouse anti-human HLA-ABC monoclonal antibody B-H9 was purchased from BioSource International (Carmarillo, CA, USA). FITC-labeled mouse IgG1 monoclonal antibody was purchased from PharMingen (San Diego, CA, USA). The relative amount of the surface MHC-peptide complexes is measured by the relative mean fluorescence intensity from FITC-labeled mouse anti-human HLA-ABC monoclonal antibody.

Acknowledgments
We are grateful to Drs R Markowitz, W Dynan, D Munn, M Iwashima and A Mellor for their critical reading of this manuscript and/or suggestions. We would like to thank H Ploegh (MIT, MA) for human TAP1 cDNA, J Trowsdale (University of Cambridge, UK) for TAP1 genomic DNA, B Seliger (Johannes Gutenberg-Universität, Germany) for anti-TAP1 antibody, D Dransfield (Medical College of

Georgia, GA, USA) for HCT116 and LST174T cell lines, B Vogelstein (Johns Hopkins University, MD, USA) for 8OS14 cell line, and A Mellor (Medical College of Georgia, GA, USA) for H2-K^b cDNA. This work is supported in part by grants from the National Institute of Health (CA76069 and CA81237) and DOD Army Breast Cancer Program (DAMD17-97-1-7019).

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