Transcriptional Regulation by p53 and p73

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The tumor suppressor p53 exerts its effect through transactivation of a wide variety of genes leading to outcomes such as cell cycle arrest or apoptosis. Both p53 protein levels and modification status are thought to play a role in its ability to discriminate between different target genes and, thereby, cell fate. Here, we have determined the contribution of p53 levels to promoter selectivity when ectopically expressed in H1299 cells. Interestingly, p53AIP1, a pro-apoptotic p53 target gene, requires a significantly higher threshold level of p53 for its activation than p21WAF1, a cell cycle arrest gene. We also found that whereas exogenous p73 exhibits similar transcriptional activity to p53 in H1299 cells, the endogenous p73 that accumulates upon DNA damage in HCT116 cells is unable to compensate for p53 function. Quantification of protein expression levels revealed that the basal expression of TAp73 in HCT116 cells is very low and, even after induction by DNA damage, it accumulates to levels that are lower than basal uninduced levels of p53. These results might partially explain why, unlike p53, p73 does not function as a major tumor suppressor.

The tumor suppressor p53, which is mutated in more than 50% of human cancer, is stabilized and activated in cells in response to genotoxic stresses or oncogenic stimuli and then initiates different cellular outcomes, including cell cycle arrest, apoptosis, or senescence (for review, see Ko and Prives 1996; Levine 1997; Bates and Vousden 1999; Prives and Hall 1999). The biological functions of p53 involve its activities as a sequence-specific transcription factor, and cellular outcomes following stress are determined through the transactivation of a wide variety of p53 target genes such as p21WAF1, MDM2, PIG3, BAX, PUMA, p53AIP1, and numerous others. Several models have been proposed to explain how the activity of p53 is regulated and how the promoter discrimination of p53 is achieved on the molecular level, leading, in some cases, to a decision between cell cycle arrest and apoptosis in response to distinct stimuli.

It has been proposed that the choice in response depends on the amount of p53 present in the cell as well as the modification state of p53 (Wang and Prives 1995; Gu and Roeder 1997; Sakaguchi et al. 1998; Oda et al. 2000; Appella and Anderson 2001). The stabilization of p53 after DNA damage is thought to occur largely through phosphorylation events that disrupt its interaction with its negative regulator, MDM2, although the literature reveals considerable complexity in that regard (Shieh et al. 1997; Prives and Hall 1999; Moll and Petrenko 2003; Poyurovsky and Prives 2006). Furthermore, some modifications, such as phosphorylation of Ser-46, may increase the affinity of p53 for a specific promoter independently of p53 expression level (Oda et al. 2000).

Analysis of target gene mRNA induction and chromatin immunoprecipitation (ChIP) assays has shown a difference in binding affinity and kinetics of p53's association with and transactivation of its target gene promoters (Szak et al. 2001; Kaeser and Iggo 2002). Although there are notable exceptions (e.g., the PUMA gene), low-affinity sites are frequently found in pro-apoptotic genes, suggesting that the level of p53 expression can determine the threshold for promoter activation and cellular outcome in some experimental conditions (Chen et al. 1996; Ludwig et al. 1996). In situ, this might permit p53, when present at low levels, to induce cell cycle arrest, allowing time to repair the DNA damage, and at higher levels (perhaps following more extensive damage), to activate the apoptotic program. It has also been shown that some proteins, for example ASPP1/2 (Samuels-Lev et al. 2001), augment the p53-dependent transactivation of only the pro-apoptotic target genes. This suggests an initial weaker binding to some pro-apoptotic promoters that, in the case of severe enough DNA damage, is augmented by p53 modification and cofactors.

The p53 family member p73 was first identified in 1997 (Jost et al. 1997; Kaghad et al. 1997). Since then, much has been discovered about this protein, but many questions remain unanswered (Irwin and Kaelin 2001a,b; Yang et al. 2002; Moll and Slade 2004). The p73 gene contains 14 exons, which, through splicing, can produce seven TA full-length isoforms that differ in their carboxyl terminus (α–η) and the corresponding ΔN isoforms. The ΔN isoforms result from transcription from an alternative promoter and have a transactivation domain different from that of the full-length isoforms. In vivo, p73α and β are the most commonly found forms, in both the TA and ΔN varieties.

The p73 protein has approximately 65% homology with p53 in its DNA-binding domain, and some homology in the transactivation and oligomerization domains (20–30% and 35–45%, respectively.) Like p53, p73 functions as a transcription factor and has been shown to transactivate a number of p53 target genes, although each protein also transactivates its own unique class of downstream targets (for review, see Harms et al. 2004). Also like p53, p73 is up-regulated in the cell following DNA damage (Agami et al. 1999; Yuan et al. 1999), although

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the mechanisms of its up-regulation are not fully understood. Finally, p73 can also exhibit modification-dependent target selectivity; for example, acetylation of p73 induces selective activation of apoptotic target genes (Costanzo et al. 2002).

Several lines of evidence challenge the role of p73 as a tumor suppressor. Whereas p53 is mutated or deleted in more than 50% of human tumors, p73 mutations in tumors are far rarer. It should be noted, however, that some tumors overexpress p73, and it is likely that it is the anti-apoptotic ΔN isoforms which are overexpressed. There is also a significant difference in phenotype between p53 and p73 knockout mice: The former are highly susceptible to tumors (Donehower et al. 1992; Attardi and Jacks 1999), whereas the latter exhibit various neurological defects but no increased tumor susceptibility (Yang et al. 2000). It cannot be ruled out, however, that this is a defect in a very specific form of apoptosis. Nonetheless, even in cell lines that express TAp73, eliminating p53 expression has a profound effect on apoptosis, further questioning

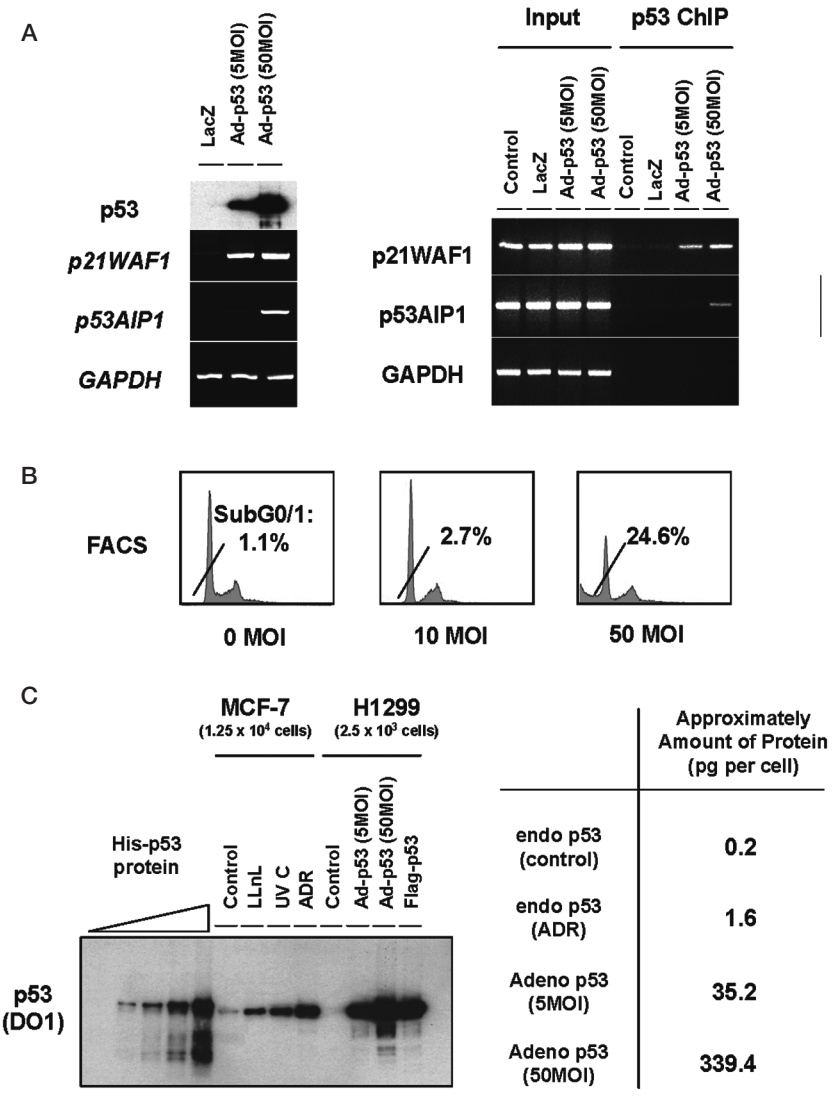

Figure 1. Differential regulation by p53 of induction of p53 target genes and binding to promoters in vivo. (*A*) Differential transactivation of p53 target genes after p53 adenovirus infection as detected by RT-PCR (*left*) and ChIP analysis (*right*). H1299 cells were transduced with p53- or LacZ-expressing adenoviruses at the indicated moi. After total mRNA was prepared, quantitative RT-PCR to detect p21, p53AIP1, and GAPDH was carried out (*left*). H1299 cells were transduced with p53 or LacZ-expressing adenoviruses at the indicated moi. Cells were treated or not with formaldehyde cross-linking. After cell lysis and sonication, p53-containing complexes were immunoprecipitated with p53 monoclonal antibodies Pab 1801 and DO-1. After reversal of cross-linking, input DNA and immunoprecipitates were determined by PCR to detect indicated promoter activity (*right*). (*B*) Differential induction of p53-dependent apoptosis by FACS analysis. H1299 cells were transduced with p53-expressing adenoviruses at the indicated moi, and collected and fixed with 70% ethanol in PBS after 36-hr infection. FACS analysis followed by PI staining was performed. (*C*) Estimation of expression level of endogenous and adeno-expressed p53 in cells. Either MCF-7 or H1299 cells were seeded at the same cell density in a 6-well plate (2.5×10^5 cells/well) and then treated with the different agents as indicated or infected with the p53 adenovirus. Cells were collected and lysed in SDS sample buffer directly and subjected to immunoblotting with p53 monoclonal antibodies (DO-1). First six lanes represent serial dilution of purified p53 recombinant protein.

the role of endogenous p73 as a tumor suppressor.

Here, we use adenoviral infection to assess whether the amount of p53 in a cell determines the protein's ability to transactivate "weaker" pro-apoptotic targets, and to determine the number of p53 molecules that are required per cell for transactivation of p21 or AIP1 promoters. Furthermore, to address the difference in apoptosis induced by endogenous p73 as compared to p53 in response to DNA damage, we have determined the levels of endogenous p73 and p53 proteins expressed in the HCT116 colorectal adenocarcinoma cell line following treatment with camptothecin.

RESULTS AND DISCUSSION

Different Levels of p53 Are Required to Transactivate Target Promoters

As mentioned above, several models have been proposed to explain how the promoter discrimination of p53 is regulated and why p53 induces cell cycle arrest under some conditions and apoptosis under others. One model postulates that p53 binds with high affinity to the promoters of cell cycle arrest genes and binds to the promoters of most pro-apoptotic genes with a low affinity. It has been shown, for example, that the p53-binding site in the p21 promoter is a much better match to the consensus sequence than the sites in some putative apoptotic target gene promoters and that the level of p53 expression determines the outcome in some experimental conditions (Chen et al. 1996; Ludwig et al. 1996).

To test whether DNA binding to select promoters is correlated with the levels of p53, we first examined both mRNA levels and chromatin binding upon expression of two different levels of p53 in H1299 cells by using different amounts (multiplicity of infection: moi) of recombinant adenovirus expressing p53 (Ad-p53). Induction of p21 and p53AIP1, a pro-apoptotic target gene, was compared by reverse-transcription polymerase chain reaction (RT-PCR) at various amounts of Ad-p53 (Fig. 1A, left panel). At a moi of 5, p53 induced p21 robustly but only barely induced AIP1, whereas at 50 moi, both p21 and AIP1 were well induced. The ChIP assay revealed that p53 at 50 moi bound both to the p21 and the AIP1 promoters, whereas at 5 moi only the p21 promoter was engaged by p53 (Fig. 1A, right panel). Consistent with these functional differences between p21 and p53AIP1, fluorescence-activated cell sorting (FACS) analysis showed that only at 50 moi of Ad-p53 was a significant level of apoptosis induced (Fig. 1B). Whereas apoptosis induced by high moi of adenovirally expressed p53 is not likely to be mediated exclusively by a single gene product, AIP1 represents a clear example of an apoptotic gene that requires a significantly higher threshold level of p53 for its activation than does a cell cycle p53 target promoter such as p21.

Next, to estimate the amount of endogenous and adenovirally expressed p53, immunoblotting was performed comparing serial dilutions of purified His-tagged human p53 recombinant protein from baculovirally infected insect SF9 cells as a standard p53 protein (Fig. 1C, left panel). In brief, either MCF-7 or H1299 cells were seeded at the same cell density (2.5×10^5 cells) and then treated with the different agents as indicated, or infected with the p53 adenovirus. Cells were lysed in SDS sample buffer directly and then subjected to immunoblotting using amounts that fall within linear range for quantification. As expected, the basal expression of endogenous p53 in MCF-7 cells was very low (~0.21 pg/cell), and p53 accumulated to 8-fold higher levels (~1.6 pg/cell) following adriamycin treatment in MCF-7 cells (Fig. 1B, right table). Interestingly, the p53 expression by Ad-p53 at 5 moi in H1299 cells was 22-fold (~35.2 pg/cell) higher than that of MCF-7 cells treated with adriamycin (Fig. 1B, right table). Adenovirally expressed p53 levels at 50 moi were 10-fold higher than at 5 moi (Fig. 1B, right table). Although we cannot exclude the possibility that posttranslational modifications of p53, such as phosphorylation and acetylation, are induced differentially at different moi, these results indicate that the levels of p53 expression determine the threshold of activation for at least some low-affinity pro-apoptotic promoters.

Comparison of Transcriptional Activities of p53 and p73

Although the DNA-binding domain of p73 interacts with the canonical p53 response element, and both p73 and p63 activate the expression of a number of p53 target genes and suppress cell growth when overexpressed, several lines of evidence indicate that although p53, p73, and p63 share structural and functional similarities and have overlapping functions, many target genes respond differentially to the different family members (Jost et al. 1997; Kaghad et al. 1997; Zhu et al. 1998; Lee and La Thangue 1999). It remains to be determined whether and how different stimuli selectively recruit one or more members of the p53 family to achieve specialized transcription responses in specific cellular contexts.

To accurately compare the transcriptional activity of p53 and p73, it is necessary to use a system where the expression levels of either p53, p73α, or p73β are normalized and regulated in the same cell line in the absence of cellular stress, since the stabilization and activation of p53 and p73 are regulated differentially after genotoxic stress through posttranslational modification, protein–protein interaction, and protein turnover. Previous work comparing the transcriptional activity of p53 and p73 is somewhat ambiguous, with some reports showing similar activity for p53 and p73β, some reporting weaker activity for p73β, and yet others showing that the difference in activity depends on the target gene. Most reports do agree that the ability of p73β to transactivate a variety of p53 target genes and to induce apoptosis is stronger than that of p73α (Lee and La Thangue 1999; Ueda et al. 1999), possibly due to the inhibitory effect of the SAM domain, located in the carboxy-terminal region specific to the alpha isoforms (Liu and Chen 2005). However, the reported difference in activity varies, with some data showing only a 2-fold difference

in activity between exogenous p73α and p73β (Ozaki et al. 1999), and a 2-fold difference in ability to induce apoptosis (Zhu et al. 1998).

To determine the transcriptional activity of p53 and p73 in our system, we used tetracycline-regulated stable H1299 clones in which the induction of HA-p53, HA-p73α, and HA-p73β can be regulated precisely without any cellular stress, and the expression of p53 is close to physiological levels (Chen et al. 1996; Zhu et al. 1998). Indeed, when expressed to the same levels, HA-p53 and HA-p73β induced p21WAF1 expression similarly (Fig. 2A). p73α was able to induce p21, but the induction level was approximately 2.5-fold less than those of p53 and p73β, as had been reported previously (Fig. 2A). To gain further information about other target genes, RT-PCR was performed to detect p21, HDM2, PIG3, and PUMA, using p53- and p73β-expressing clones (Fig. 2B). The extent of transactivation was similar for all four target genes by p53 and p73β, even when their expression was down-regulated to low levels using tetracycline. These results suggest that p73 is likely to have transcriptional activity similar to that of p53 on several p53 target genes when the expression level is controlled without any cellular stresses.

Next, to examine and compare the transcriptional activity of endogenous p53 and p73, HCT116 cells which either express p53 (HCT-$p53^{+/+}$) or lack p53 (HCT-$p53^{-/-}$) were characterized. In both of these cell lines, as we have reported previously, the major p73 isoform expressed is TA-p73α, although TA-p73β has also been detected (Lin et al. 2004; Urist et al. 2004). Cells were treated with camptothecin (CPT), 5-fluorouracil (5-FU), or daunorubicin (Dauno) to induce p53 and p73 expression, and then western blot, RT-PCR, and FACS analyses were performed (Fig. 3). Both p53 and p73 were up-regulated following treatment with CPT and Dauno, whereas only p53 levels were increased following 5-FU treatment, demonstrating that p53 and p73 are regulated in distinct ways upon different stimuli (Fig. 3A).

Although p21 mRNA and protein levels increased significantly following DNA damage in the $p53^{+/+}$ cells, no or little induction of p21 was observed after CPT or Dauno treatment in the $p53^{-/-}$ cells, even though there was a marked increase in detectable p73, suggesting either that levels of endogenous p73 induced by DNA damage were not enough to activate p21 in HCT116 cells lacking p53 or that p73 activity is somehow repressed in these cells. Furthermore, although both cell lines exhibited changes in cell cycle distribution following DNA damage, a significantly greater sub-G_0/G_1 population was observed in the $p53^{+/+}$ cells after CPT and 5-FU treatment (Fig. 3B). Consistent with the induction of p21, G_1 arrest was not observed after CPT or Dauno treatment in $p53^{-/-}$ cells (Fig. 3B, lower panel). In addition, the $p53^{-/-}$ cells did undergo G_1 arrest after 5-FU treatment but, because there was no p73 induction under these conditions, the p73 dependence of these cellular outcomes is questionable.

To confirm the binding ability of endogenous p73 to a target promoter in HCT116 cells, a p73 ChIP assay was performed using an anti-p73 monoclonal antibody. The endogenously expressed TA-p73α that accumulated after CPT treatment was associated with the p21WAF1 promoter in both $HCT116^{+/+}$ and $HCT116^{-/-}$ cells, suggesting that p73 is not functionally impaired in its ability to bind DNA in HCT116 cells (data not shown).

Taken together, our results indicate that whereas exogenous p73 exhibits similar transcriptional activity to p53, the endogenous p73 that accumulates upon DNA damage is unable to compensate for p53 function. Thus, the induction of endogenous p73 does not lead to cellular outcomes such as p21 induction, G_1 arrest, or apoptosis in HCT116 $p53^{-/-}$ cells.

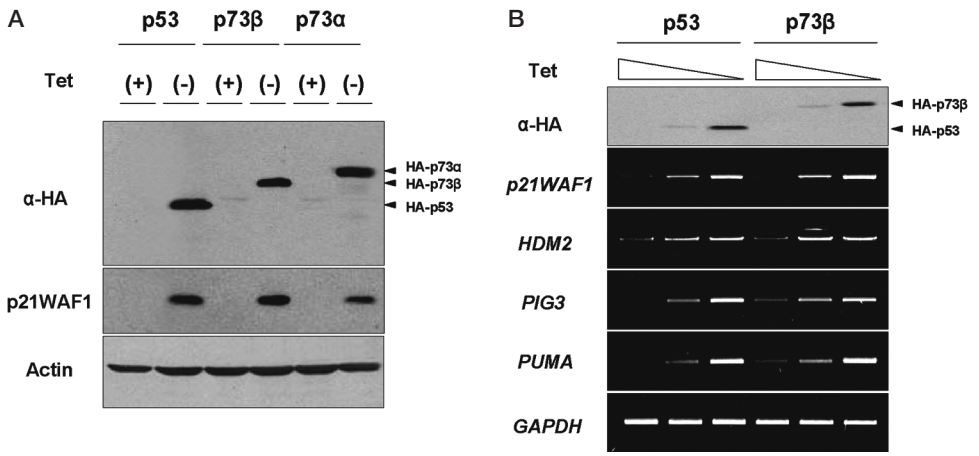

Figure 2. Induction of target genes by p53 and p73. (*A*) HA-p53, HA-p73α, or HA-p73β expression in tetracycline-regulated H1299 clones was induced by removal of tetracycline. To determine p21 protein induction by p53 and the p73 isoforms, HA-p53, HA-p73α, and HA-p73β levels were induced and detected by immunoblotting with anti-HA antibody. p21 expression was determined by anti-p21 monoclonal antibody (Oncogene Ab-1), and actin (Sigma) was used as loading control. (*B*) To assess the induction of several target genes by p53 and p73β, high and low protein expression level was regulated by tetracycline and visualized by immunoblotting (*top panel)*. mRNA levels of p53 target genes, p21, HDM2, PIG3, PUMA, and as an internal control mRNA, GAPDH, were assessed by quantitative RT-PCR.

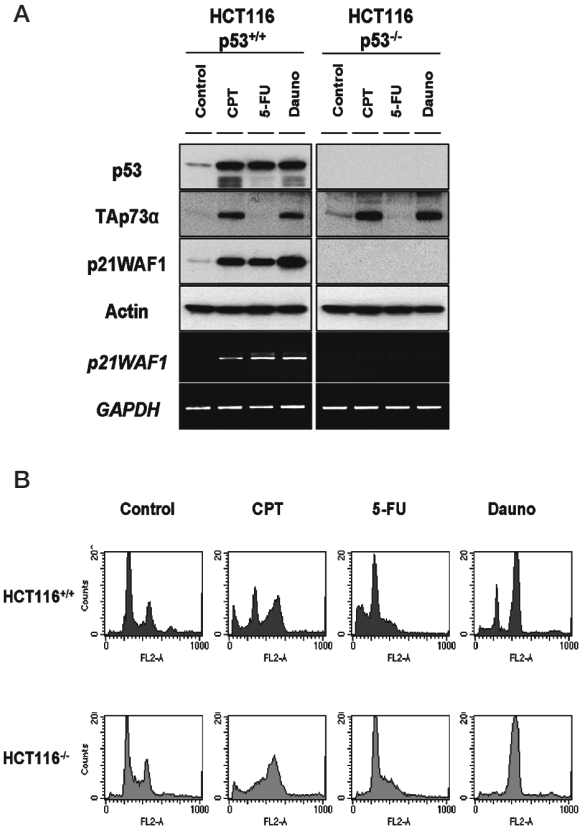

Figure 3. p73 in HCT116 cells does not detectably induce p21WAF1 expression or affect cell cycle distribution. (*A*) p53 and p73 expression was induced in $p53^{+/+}$ and $p53^{-/-}$ HCT116 cells by treatment with CPT (300 nM), 5-FU (488 μM), or Dauno (200 nM) for 24 hr (for immunoblotting or RT-PCR) or 48 hr (for flow cytometry). Western blotting was performed to detect p53 using DO-1, p73 using a TA-p73 polyclonal antibody, and p21WAF1 (Oncogene, Ab-1). As a loading control, actin was measured by anti-actin antibody (Sigma). Quantitative RT-PCR was then performed to assess the induction of p21WAF1 mRNA. (*B*) To determine the cell cycle profile, cells were collected and fixed with 70% ethanol 48 hr after drug treatment. After PI staining, FACS analysis was performed.

How Much p53 and p73 Protein Is Present in HCT116 Cells?

Returning to our findings that, whereas p73α and β induced several p53 target genes including p21 when expressed at comparable levels to p53 in H1299 cells, endogenous p73 could not induce p21WAF1 in $HCT116^{-/-}$ cells, we considered the possibility that there is a threshold amount of protein required to induce p53 target genes, and endogenous p73 levels after DNA damage were not enough to induce p21 in HCT116 cells. Therefore, we determined the amount of endogenous p53 and p73α protein in HCT116 cells. Because each antibody differs in its sensitivity in detecting its respective protein by immunoblotting, we used protein standards of purified HA-tagged p53 or p73α. To this end, HA-p53 and HA-p73α recombinant proteins were affinity-purified from baculovirally infected SF9 cells and then subjected to SDS-PAGE followed by silver staining (Fig. 4A, upper right panel). Quantification was also performed using a bovine serum albumin (BSA) standard curve, and concentration of each stock solution is shown (Fig. 4A, left upper panel). Indeed, the signal intensity of these two proteins using anti-HA antibody (normalized by molar ratio) supports the quantification obtained by BSA standard in silver staining.

Next, to determine the amount of endogenous p53 and TA-p73α expressed in HCT116 cells, we made serial dilutions of the purified HA-p53 and HA-p73 proteins and compared them to the levels of endogenous protein induced following CPT treatment by immunoblotting using the anti-p53 monoclonal antibody (DO-1) and anti-p73 polyclonal antibody (TA-p73) (Fig. 4B). This allowed us to estimate the basal expression of endogenous p53 as $\sim 5 \times 10^{-4}$ pmole per microgram, whereas p53 levels reach 111.48×10^{-4} pmole per microgram of whole-cell extract (WCE) after CPT treatment (23 ng/μg of WCE). Thus, p53 accumulated 20-fold following DNA damage (Fig. 4B, left panel).

The basal expression of TA-p73α was too low to detect in this range of protein (probably less than 0.287×10^{-4} pmole per microgram of WCE; 2 ng/μg of WCE) (Fig. 4B; see table). In addition, the amount of endogenous TA-p73α expressed following DNA damage was in the range of that of uninduced p53, and about 30-fold lower than that of p53 following CPT treatment, suggesting that the endogenous p73α expression level is much lower than that of p53 in the presence or absence of DNA damage in HCT116 cells. Thus, it is possible that the inability of endogenous p73 to replace p53's function is, at least in part, due to the significantly lower levels of p73 in the cell.

Currently, it is thought that the choice between cell cycle arrest and apoptosis is determined in part by levels of p53 and in part by its modification status. In this work, we address this issue by using adenoviral infection to determine whether p53 levels in a cell correlate with its ability to transactivate pro-apoptotic targets. Indeed, the levels of p53 expression determined the threshold of activation for the low-affinity pro-apoptotic AIP1 promoter. Interestingly, a 10-fold difference in p53 levels could determine not only whether the pro-apoptotic AIP1 promoter was transactivated, but also whether binding at the AIP1, but not p21WAF1, promoter could be detected, suggesting a threshold at the level of DNA binding. Furthermore, we calculated the average number of p53 molecules that are required per cell for transactivation of p21 or AIP1 promoters. Interestingly, endogenous p53 is present in cells at levels below the apoptotic threshold calculated for adenovirally expressed p53, which raises a number of questions about the difference between endogenous and exogenous proteins and their ability to induce apoptosis. It should be noted as well that a number of experiments have indicated that endogenously expressed p73 is not inert. For example, siRNA knockdown of p73 in SW480 cells (Irwin et al. 2003) or H1299 cells (Urist et al. 2004) reduces the low level of apoptosis caused by some agents in the absence of p53. Perhaps HCT116 cells are particularly defective in responding to p73 induction. Nevertheless, ChIP analysis in these cell lines shows that endoge-

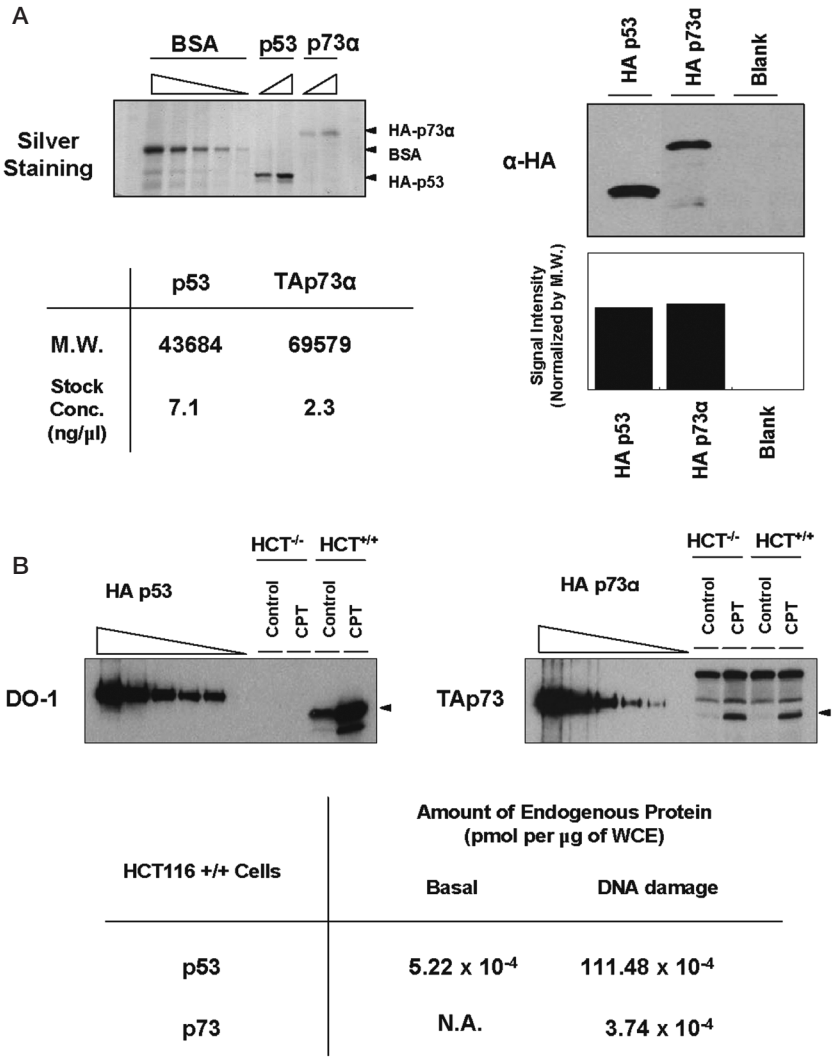

Figure 4. Quantification of expression levels of endogenous p73 and p53 after CPT treatment. (*A*) Purified p53 and p73 protein standard was obtained by affinity-purifying HA-p53 and HA-p73α baculovirally expressed in SF9 cells. To determine the nanogram amounts of each protein, a silver stain was performed, with BSA as control (*top left panel*). The quantification is presented in the bottom left panel. To assure the accuracy of our quantification, we performed a western blot using normalized amounts of the protein standards (*right top panel*). As shown in the bottom right panel, the p53 and p73 protein standards have similar amounts of protein. (*B*) To determine the amounts of endogenous p53 and p73 expressed in HCT116 cells, 80 μg of lysate from uninduced cells and cells treated with CPT was loaded onto an SDS-PAGE gel along with serial dilutions of the p53 or p73 protein standards. Immunoblotting was then performed using DO-1 antibody to detect p53 (*top right*) and TA-p73 polyclonal antibody to detect p73 (*top left*). The picogram and picomolar quantifications of p53 and p73 are presented in the bottom panel.

nous TA-p73α is bound to the p21 promoter, suggesting that this protein does have DNA-binding activity. Experiments with siRNA would be required to show whether the endogenous p73 has any transcriptional or pro-apoptotic activity, since, although unlikely, it is possible that this protein can bind promoters but cannot transactivate them in HCT116 cells. It would also be important to confirm whether ectopic p73 expression can induce p53 target genes and apoptosis in HCT116 cells.

The p53 family member p73, when first discovered, was postulated to be a tumor suppressor, largely based on the highly conserved functional activity between the two family members. However, data from both human tumors and p73 knockout mice brought the tumor suppressor function of this p53 family member into question. In screening a few cell lines for levels of p73, we have found that HCT116 cells are among those that are relatively more abundant in this protein. Therefore, our quantification of the levels of p73 present in these cells may at least partially explain the failure of cells to undergo significant arrest or apoptosis in the absence of p53.

Most published work shows that exogenous p53 and p73β have similar transcriptional activities, and p73α is somewhat weaker than p73β. We reproduced these findings using a tetracycline-regulated system where ectopic protein expression can be induced in the absence of DNA

damage. Our data indicate that p53 and p73β do exhibit almost identical activity in initiating transcription of four canonical p53 target genes. We also found that p73α induced about three times less p21 protein expression than did p53 or p73β. Although we did not measure the effect of the overexpression of these proteins on cell cycle distribution, several lines of evidence suggest that the two family members can induce comparable levels of apoptosis. It is therefore likely that the inability of p73 to induce a p53-like cell cycle profile is because the levels to which p73 is expressed are insufficient. The quantification of p73 expressed in HCT116 cells compared to p53 shows that the levels of the former are almost 30 times lower than that of the latter. Such low levels of p73 (in addition to the already lower activity of the alpha isoform) could explain why this protein does not phenocopy p53. On the basis of these data, we would predfict that if it were expressed at levels similar to p53, endogenous p73 should be able to transactivate target genes and induce cell cycle arrest and apoptosis to an extent similar to p53.

Considering that splicing variants of p73 display different transcriptional properties and biological functions, and that they are expressed differentially among normal human tissues and cell lines, further investigation using other cell lines will be required to examine the relation between the levels and activities of p73 or p53 and the threshold for target gene transactivation and induction of apoptosis. Taken together, although we cannot exclude the possibility of cell-type specificity and isoform-specific functions, our results indicate that compared to p53, TA-p73 is expressed at very low levels in cells, which may explain in part why p73 does not function as a tumor suppressor as does p53.

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