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Inhibition of HIF-1- and wild-type p53-stimulated transcription by codon Arg175 p53 mutants with selective loss of functions

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Overexpression of ectopic mutant p53 represses wild-type p53-stimulated transcription, known as a dominant negative effect. On the other hand, overexpression of wild-type p53 can repress transcription stimulated by several transcription factors, including hypoxia-inducible factor-1 (HIF-1). Using a panel of well-characterized Arg175 p53 mutants we found that only mutants (Tyr175, Trp175, Asp175 and Phe175) which have completely lost their ability to transactivate repress wild-type p53-stimulated Bax, p21 and PG13 promoter constructs. In contrast, Asn175, Gln175, Leu175 and Pro175 mutants which partially retained transactivating functions did not exert dominant negative effects against PG13 and p21 promoter constructs. However, these latter mutants failed to activate Bax and, instead, exerted a dominant negative effect on a Bax-Luc promoter construct. We conclude that a dominant negative effect is promoter selective as a consequence of selective loss of transactivating function. Albeit less potent than wild-type p53, all Arg175 p53 mutants retained partial ability to repress HIF-1-stimulated transcription. We propose that transrepression and the dominant negative effect have similar mechanisms and may involve competition with transcription factors (wild-type p53, HIF-1, etc.) for cofactors such as p300. Thus, a p53(22/23) mutant, which is deficient in p300 binding, did not exert dominant negative effects. Like transrepression, the dominant negative effect required overexpression of mutant p53 and, therefore, is not dominant. In the presence of a wild-type p53 allele, levels of endogenous mutant p53 protein were low in heterozygous cells. Endogenous mutant p53 became overexpressed only after loss of the second p53 allele. Therefore, endogenous mutant p53s are unable to display a dominant negative effect. This explains why loss of the second p53 allele is required to eliminate p53 functions in cancer cells.

Introduction

Wild-type p53 is a transcription factor that binds to specific sequences in DNA and transactivates numerous genes, including p21, Bax and Mdm-2 (1–5). Mutations in the p53 tumor suppressor are the most common alterations in human tumors. Mutations in p53 can result in loss of its functions and in acquisition of dominant negative properties characterized by inhibition of wild-type p53-stimulated transcription (6–8). However, some observations suggest that mutant p53 is not dominant (9–12) and most human tumors have an inactive second p53 allele (13,14). One current model proposes that mutant p53 exerts its dominant-negative effect by driving wild-type p53 into a mutant conformation (15). However, several observations argue against the ability of mutant p53 to change the conformation of wild-type p53 because mutant p53 proteins having a wild-type conformation are still able to exert dominant negative effects (16). Furthermore, mutant p53 can inhibit transcriptional activity of wild-type p53 without heterooligomerization (17).

When overexpressed wild-type p53 blocks transcription from numerous promoters lacking p53-binding sites (18–24), including transcription that is stimulated by hypoxia inducible factor-1 (HIF-1). Under hypoxic conditions HIF-1 activates transcription of genes encoding glucose transporters, glycolytic enzymes and vascular endothelial growth factor in order to facilitate metabolic adaptation to hypoxia (25,26). HIF-1 is essential for tumor vascularization and development (27). As previously shown for wild-type p53 (22,28), we found that some mutant p53s can inhibit HIF-1-stimulated transcription. Transrepression by p53 may be a result of competition with other transcription factors for co-activators. In this scenario p53s would most strongly compete with themselves (e.g. wild-type with mutant) for any co-activators. This predicts that mutant p53 that has lost its ability to transactivate but is able to transrepress will inhibit wild-type p53-stimulated transcription.

Here we have used a previously well-characterized panel of codon 175 (Arg) p53 mutants (21,29). Codon 175 in p53 is one of the hot-spots for mutation in human cancer, because this residue plays an important role in maintaining the structure of the DNA-binding domain (30,31). We compared the ability of these mutants to activate p53-responsive promoters (transactivation) with their ability to inhibit p53-dependent transcription in cells with wild-type p53 (dominant negative effect) and their ability to inhibit HIF-1-stimulated transcription (transrepression). We demonstrate that the loss of transactivating function by mutant p53 coupled with a certain ability to transrepress is sufficient and required to exert a negative effect. We demonstrate that the dominant negative effect is promoter selective, a phenomenon which was previously unrecognized but can be explained if one proposes that transrepression (inhibition of a heterologous promoter) and dominant negative activity (inhibition of a homologous promoter) have similar mechanisms. Since the loss of transactivating function is promoter specific, the dominant negative effect is also promoter specific.

Materials and methods

Cell lines and reagents

Two breast cancer cell lines, MCF-7 (wild-type p53) and SKBr3 (mutant p53), and two prostate cancer cell lines, LNCaP (wild-type p53) and PC3M

Abbreviations: DFX, desferrioxamine; HIF-1, hypoxia inducible factor-1.

(p53-null), were obtained from ATCC and incubated in RPMI-1640 medium and 10% fetal bovine serum. A2780 (1A9) is a single cell clone of the human ovarian carcinoma cell line A2780. Epothilone-resistant subclones, designated A4 and A8, were isolated by exposure to 6 ng/ml epothilone A. PTX22, a paclitaxel-resistant clone, was initially isolated as individual clones in a single step selection, by exposing 1A9 cells to 5 ng/ml paclitaxel in the presence of 5 μg/ml verapamil, a Pgp antagonist. After initial expansion in 5 ng/ml paclitaxel, the concentration of paclitaxel in the medium was gradually increased to 15 ng/ml. Adriamycin was obtained from Sigma and dissolved in DMSO as a 2 mg/ml stock solution. Epothilone A was obtained from Dr Frank Lee (Bristol Myers, Princeton, NJ) and paclitaxel was from Bristol-Myers.

p53 expression vectors and p53-responsive and HIF-1-responsive promoters

Ad-p53, a wild-type p53-expressing replication-deficient adenovirus, was obtained from Dr B.Vogelstein (Johns Hopkins University). Viral titer was determined as previously described (32). Multiplicity of infection is defined as the ratio of total number of viruses used in a particular infection to the number of cancer cells to be infected (i.e. number of viruses per cell). Bax-Luc, a Bax promoter–luciferase construct was previously described (21). WWP-Luc, a p21 promoter–luciferase construct, and PG13-Luc, containing a generic p53 response element, were obtained from Dr B.Vogelstein. The control luciferase plasmid, pGL2-Control, driven by the SV40 promoter and enhancer sequences, was purchased from Promega (Madison, WI). PCMV-galactosidase was purchased from Clontech (Palo Alto, CA). Arg175 mutants were described previously (21,29). The p53 double point mutant p53(22/23) was described previously (33).

HRE-Luc, a HIF-responsive construct that encompasses the hypoxia-responsive element of the iNOS promoter, was provided by Dr G.Melillo (NCI, NIH, Bethesda, MD) and was described previously (34,35). The pCMVβ.HA-HIF-1α expression plasmid was obtained from D.Livingston (Dana Farber Cancer Institute, Boston, MA). PcDNA3.1 was obtained from Invitrogen (Carlsbad, CA). pCMV-Luc was prepared by subcloning the CMV promoter (digested with *Nhe*I and *Hin*dIII from pcDNA3.1) in the PGL3B vector (Invitrogen).

Transient transfection assay

Aliquots of 50 000 cells were plated in 24-well plates (Costar, Acton, MA). The next day the cells were transfected with plasmids in the presence of

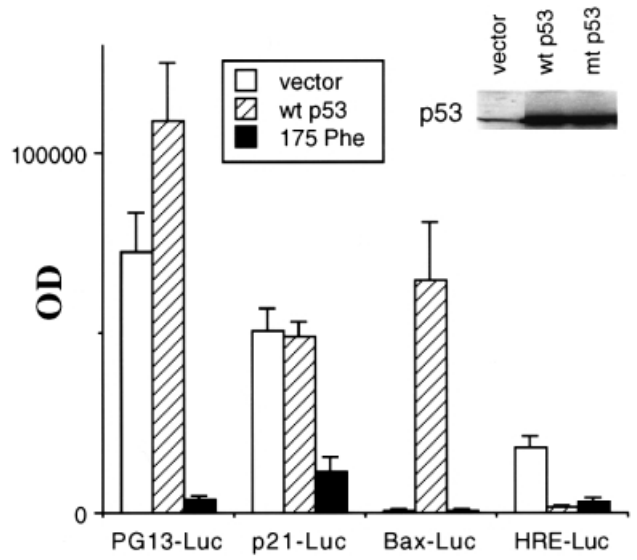

Fig. 1. Effects of wild-type p53 and dominant negative mutant p53 on p53- and HIF-1-responsive promoter constructs. MCF-7 cells were co-transfected with 0.25 μg either p53-responsive (PG13-Luc, p21-Luc, Bax-Luc) or HIF-1-responsive (HRE-Luc) reporter and with 0.25 μg either wild-type p53, Phe175 or empty vector. After 30 h cells were lysed and luciferase activity was measured as described in Materials and methods. The expression of luciferase is shown as a percentage of expression for cells co-transfected with empty vector (control). The results represent the means of three transfections.

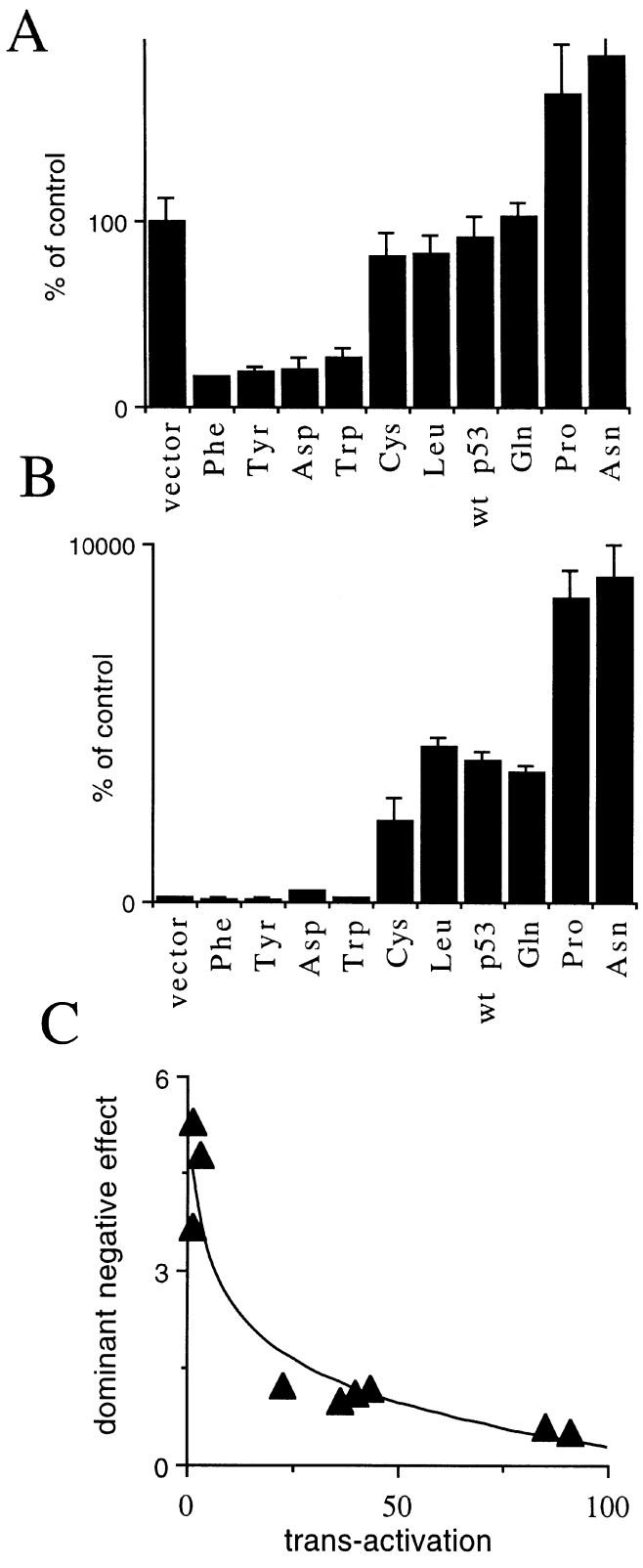

Fig. 2. Lack of transactivation correlates with a dominant negative effect. (**A**) Dominant negative effect. Repression of PG13-Luc in MCF-7 cells which possess an endogenous wild-type p53. Results are expressed as a percentage of the vector control, set at 100%. Cells were co-transfected with PG13-Luc and either a p53-expressing construct or empty vector (control). Following 20 or 36 h cells were lysed and luciferase activity was determined as described in Materials and methods. (**B**) Transactivation. Stimulation of PG13-Luc expression in p53-null PC3M cells. Results are expressed as a percentage of the vector control, set at 100%. (**C**) Correlation between transactivation (B) and dominant negative effect [calculated as 1/OD (%) and shown in (A)].

Lipofectamine (Gibco BRL, Gaithersburg, MD). If indicated cells were transfected with TransFast Transfection Reagent (Promega) according to the manufacturer's recommendations. After 3–6 h incubation with the plasmid–lipid suspension in OptiMeM medium the medium was changed and cells were grown for an additional 16–36 h, if indicated under hypoxic conditions (1% O_2), then cells were lysed and analyzed for luciferase activity. In some experiments, when anoxia-mimicking conditions instead of hypoxia were used, cells were incubated with 160 μM desferrioxamine (DFX) as previously described (36).

Immunoblot analysis

Cells were lysed in TNES buffer (50 mM Tris–HCl, pH 7.5, 2 mM EDTA, 100 mM NaCl, 1 mM sodium orthovanadate, 1% NP40) containing protease inhibitors (20 μg/ml aprotinin, 20 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride). Proteins were resolved by 8% SDS–PAGE for the detection of p53 or by 12.5% SDS–PAGE for the detection of p21, as previously described (37). Immunoblotting for p53 and p21 was performed using antibodies from Oncogene Research (Calbiochem). Blots were developed by incubation with peroxidase-labeled secondary antibodies followed by chemiluminescent peroxidase substrate (Dupont).

Results

Dominant negative effect, transactivition and transrepression by p53

We used p53-inducible constructs with the $p21^{WAF1/CIP1}$ (p21-Luc) or Bax (Bax-Luc) promoters and also a construct with a p53 consensus binding site repeated 13 times (PG13-Luc) (3,38). MCF-7 cells which have endogenous wild-type p53 express PG13-Luc and p21-Luc at high levels, which therefore cannot be significantly further induced by exogenous wild-type p53. However, MCF-7 cells express Bax-Luc at low levels (Figure 1). Co-transfection with wild-type p53 stimulated Bax-Luc, demonstrating that while basal levels of wild-type p53 are sufficient to transactivate p21-Luc and PG13-Luc, higher levels are required to activate the Bax promoter construct. In contrast, co-transfection with mutant p53 (Phe175) inhibited both PG13-Luc and p21-Luc. This inhibition of p53 transcription by mutant p53 is referred to as a dominant negative effect. Importantly, both wild-type p53 and Phe175 inhibited HIF-1-stimulated transcription (HRE-Luc). Phe175 did not stimulate any of the p53-responsive constructs (Figure 1).

Loss of transactivating function correlates with a dominant negative effect

As shown above, the dominant negative effect of Phe175 was associated with loss of its transactivating function, but their dominant negative abilities have never been tested. To further examine the relationship of these two properties we compared the dominant negative effects (Figure 2A) and transactivating function (Figure 2B) of a panel of p53 proteins with mutations at residue 175. Dominant negative properties were evaluated in the presence of endogenous wild-type p53 in MCF-7 cells by co-transfection of each mutant p53 with PG13-Luc (Figure 2A). Transactivition was evaluated by co-transfection of PG13-Luc with the p53 mutants in p53-null PC3M cells (Figure 2B).

Dominant negative effects were detected by transfection of MCF-7 cells with Phe175, Tyr175, Trp175 and Asp175 (Figure 2A). These and only these mutants had lost their transactivating function (Figure 2B). We plotted the ability of mutant p53s to transactivate PG13-Luc in PC3M versus their abilities to exert a dominant negative effect. Mutants lacking transactivation showed the highest dominant negative effects. Therefore, the dominant negative effect and loss of transactivation function were correlated (Figure 2C).

The dominant negative effect is promoter specific

To evaluate whether loss of transactivation function and the dominant negative effect are promoter selective we performed experiments using a Bax promoter construct which differs from other p53-responsive promoters in two ways. First, as shown in Figure 1, endogenous wild-type p53 was not sufficient to activate Bax-Luc, which was activated by overexpressed wild-type p53. Second, it is not activated by some p53 mutants which otherwise activate p21-Luc (21,39). Pro175 and Asn175 possessed full ability to transactivate PG13-Luc and p21-Luc (Figure 2 and data not shown) but they did not inhibit wild-type p53-stimulated PG13-Luc or p21-Luc (no dominant negative effect). However, Pro175 and Asn175 mutants, like Phe175, were very poor transactivators of Bax-Luc (Figure 3A). We next evaluated the dominant negative effects of these mutants in the presence of 0.025 μg wild-type p53-expressing plasmid that allows Bax-Luc transactivation (Figure 3B). Cells were transfected with Bax-Luc plus 0.025 μg CMVwtp53 and co-transfected with an excess (1:20) of p53-expressing constructs (Phe175, Pro175 and Asn175). When their ability to inhibit Bax-Luc (dominant negative effect) was examined, like Phe175, both Pro175 and Asn175 were able to inhibit wild-type p53-stimulated Bax-Luc (Figure 3B). Consistent with this, Phe175, which was least able to transactivate Bax-Luc (Figure 3A), had the most potent dominant negative effect (Figure 3B). Thus, loss of transactivation function was coupled with the emergence of dominant negative effects.

Dominant negative mutants can repress HIF-1-stimulated transcription

Wild-type p53 can transrepress several cellular and viral promoters, including those with a HIF-1-binding site. As shown in Figure 1, dominant negative Phe175 was able to transrepress HIF-1-stimulated transcription. We next investigated other p53 mutants. All mutants were able to transrepress HRE-Luc transcription (Figure 4). Wild-type p53 and Cys175 were the most potent repressors, with Gln175 the least potent. Transcriptionally inactive and dominant negative Tyr175, Trp175 and Asp175 possessed intermediate activity. Similar results were obtained in all three cell lines examined, MCF-7, PC3M and SKBr3, indicating that this effect was independent of the endogenous p53 status.

Comparison of transrepression and dominant negative effects of mutant p53

We next compared the ability of wild-type p53 and transcriptionally inactive p53 mutants to transrepress HRE-Luc under normoxic (low basal HIF-1 activity) versus hypoxic conditions (high HIF-1 activity). Trp175 and other p53s were able to inhibit basal as well as induced HRE-Luc activity (Figure 5A and data not shown). Basal HIF-1-dependent transcription (Figure 5A, open bars) was significantly inhibited by Trp175 (Figure 5A). As expected, HIF-1-dependent transcription was dramatically stimulated by introduction of ectopic HIF-1 (Figure 5A). Under this condition HIF-1 induced HIF-1-dependent transcription (HRE-Luc expression) even in the presence of Trp175 (Figure 5A). The observation that HIF-1 could partially overcome p53-mediated transrepression was extended by performing similar experiments using DFX to chemically induce endogenous HIF-1 protein (36).

Furthermore, just as transrepression of HRE-Luc by p53 could be partially abrogated by overexpression of HIF-1 (Figure 5A), the dominant-negative effect of mutant p53 (Trp175) could be dampened by overexpression of wild-type

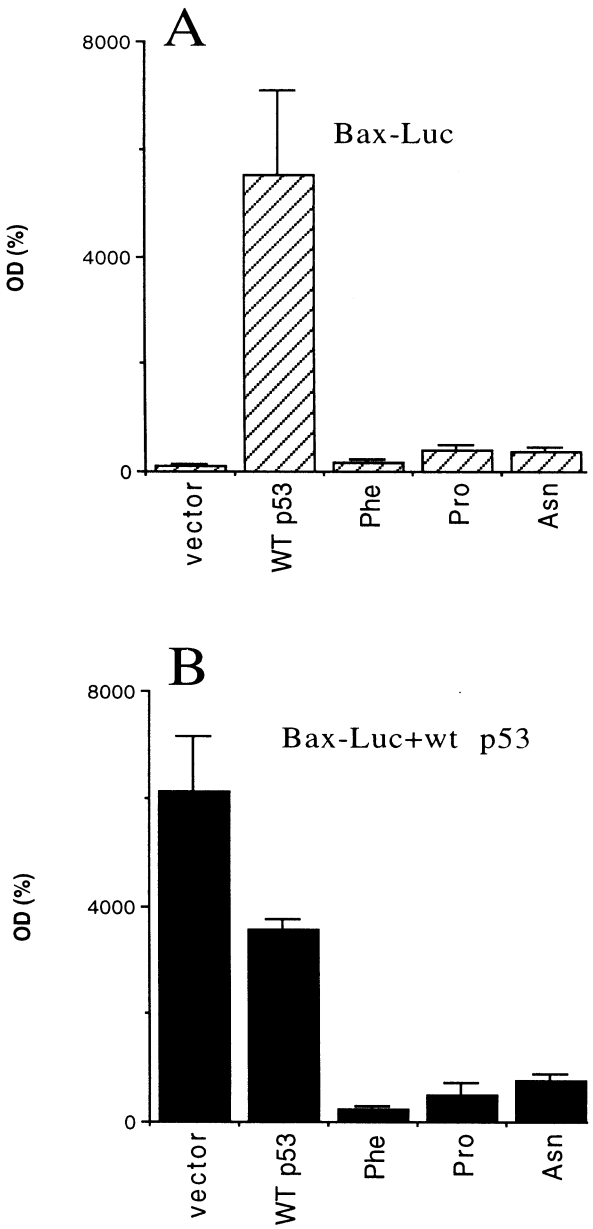

Fig. 3. Failure to transactivate Bax-Luc by otherwise transcriptionally active mutants leads to a selective dominant negative effect on the Bax promoter. (**A**) Transactivation. Stimulation of Bax-Luc expression. Cells were co-transfected with 0.25 μg Bax-Luc and 0.25 μg p53-expressing construct or empty vector (control). After 20 h cells were lysed and luciferase activity was determined as described in Materials and Methods. The results represent the means ± standard deviation of three experiments. The results with the vector control were assigned a value of 100%. (**B**) Dominant negative effect. Repression of Bax-Luc expression was determined in the presence of 0.025 μg wild-type p53-expressing plasmid that caused Bax-Luc activation. Cells were transfected with 0.25 μg Bax-Luc plus 0.025 μg CMVwt p53 and co-transfected with an excess (0.5 μg) of p53-expressing construct (wild-type p53 or the Phe175, Pro175 or Asn175 mutant) or empty vector (control). After 20 h cells were lysed and luciferase activity was determined as described in Materials and methods. The results represent the means ± standard deviation of three experiments. Results are expressed relative to the results with the vector alone in the absence of exogenous wild-type p53.

p53 (Figure 5B). For example, the dominant negative effect of Trp175 was partially abolished by exogenous wild-type p53 or when endogenous wild-type p53 was induced by adriamycin, a DNA-damaging drug (Figure 5B).

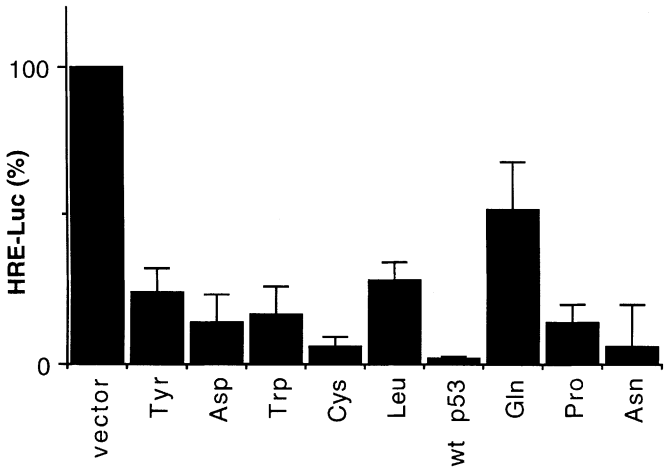

Fig. 4. Repression of HIF-dependent transcription by p53 mutants. Transrepression of HRE-Luc expression by p53-expressing constructs. PC3M and MCF-7 cells were transfected with HRE-Luc and co-transfected with p53-expressing constructs and then exposed to hypoxia for 16 h. Mean values obtained in these two cell lines are shown. Results are expressed relative to the vector control results, which were set at 100%.

Indeed, the extent of dominance is determined by the excess of mutant p53. Therefore, when SKBr3 cells with endogenous mutant p53 were transfected with wild-type p53 a 60- to 70-fold induction of expression of PG13 and Bax promoter constructs was observed (Figure 6A). Furthermore, endogenous p21 protein was dramatically induced by wild-type p53 and Pro175 in SKBr3 cells (Figure 6B). Since the transfection efficiency of SKBr3 cells did not exceed 10%, transfected p53s cannot be seen against the background of mutant endogenous p53. We next tested p53(22/23), a p53 double point mutant at residues 22 and 23 in the N-terminal domain, which is required for binding p300/CBP transcriptional co-activators (33,40). Both HIF-1α and p53 bind to p300 and p300 is required for the transcription functions of both HIF-1α and p53 (40–44). Therefore, the p53(22/23) mutant did not inhibit HIF-1-dependent transcription (22), did not activate transcription and did not induce p21 protein (Figure 6B). Importantly, despite loss of transactivating functions, this mutant p53 did not exert a dominant negative effect (Figure 6C). Thus, the 22/23 mutant lacks both dominant negative and transrepressing effects.

Lack of a dominant negative effect in heterozygous cells

The current notion that mutant p53 is dominant over wild-type p53 is principally based on transfection of exogenous mutant p53 into cells with low levels of endogenous wild-type p53.

We isolated two epothilone-resistant clones, designated A4 and A8, by exposing parental A2780 (1A9) cells to epothilone A (45). Whereas parental cells contained two wild-type p53 alleles, these two clones are heterozygous for p53 and harbor a single mutation at residue 245 (GGC→GAC, Gly→Asp), as determined by sequence analysis (data not shown). If mutant p53 is dominant one would predict: (i) undetectable wild-type p53 activity as measured with p53-responsive plasmids; (ii) high basal levels of p53 protein and therefore no further p53 induction following DNA damage.

Using either PG13-Luc (Figure 7A) or p21-Luc (data not shown) we found high levels of wild-type p53 activity in both heterozygous clones. As expected, p53-responsive plasmids were not expressed in clone PTX22 (Figure 7A), which lacks

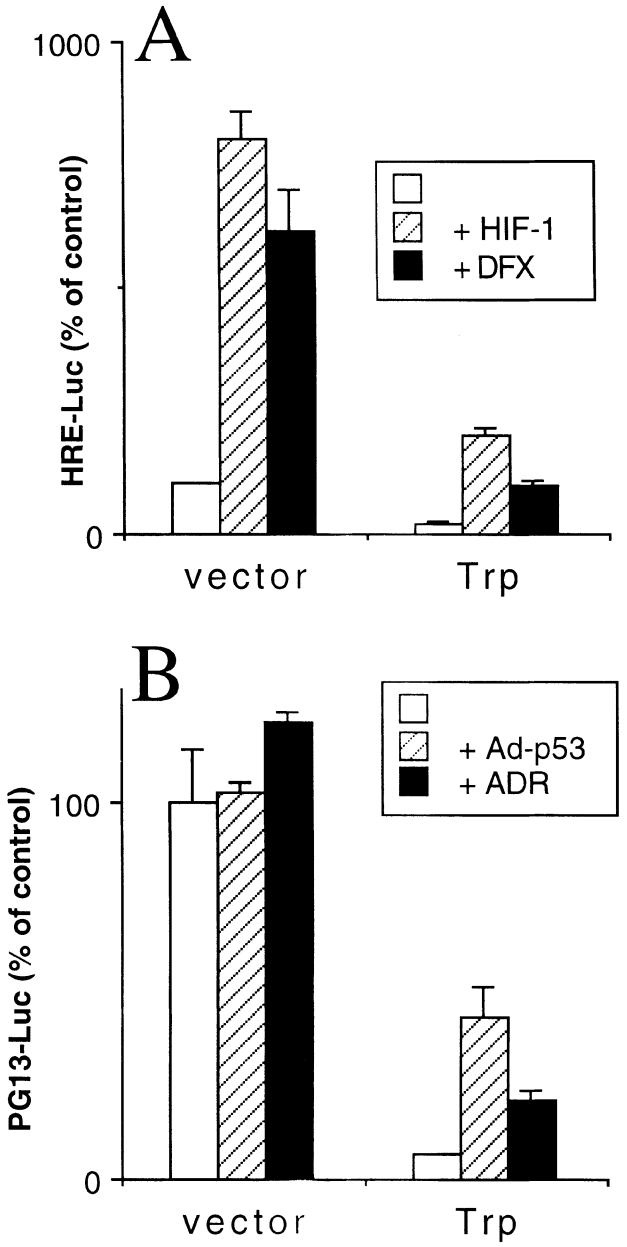

Fig. 5. Comparison of transrepression and dominant negative effect. (**A**) HIF-1 induction by either introduction of ectopic HIF-1 or by DFX partially abrogates repression of HIF-1-stimulated transcription by Trp175. MCF-7 cells were co-transfected with a HIF-1-responsive plasmid–Luc construct, HRE-Luc, and either Trp175 or the empty vector. + HIF-1, cells were co-transfected with CMV-HIF-1α to overexpress HIF-1; +DFX, cells were treated with 260 μM DFX to induce endogenous HIF-1. The untreated control vector result was assigned a value of 100%. (**B**) Wild-type p53 overexpression following infection with Ad-p53 partially blocks the dominant negative effect of Trp175. MCF-7 cells were co-transfected with a p53-responsive construct, PG13-Luc, and either Trp175 or the empty vector. + wild-type p53, cells were infected with Ad-p53 to overexpress wild-type p53; + ADR, cells were treated with 200 ng/ml adriamycin to induce endogenous wild-type p53. The untreated control vector result was assigned a value of 100%.

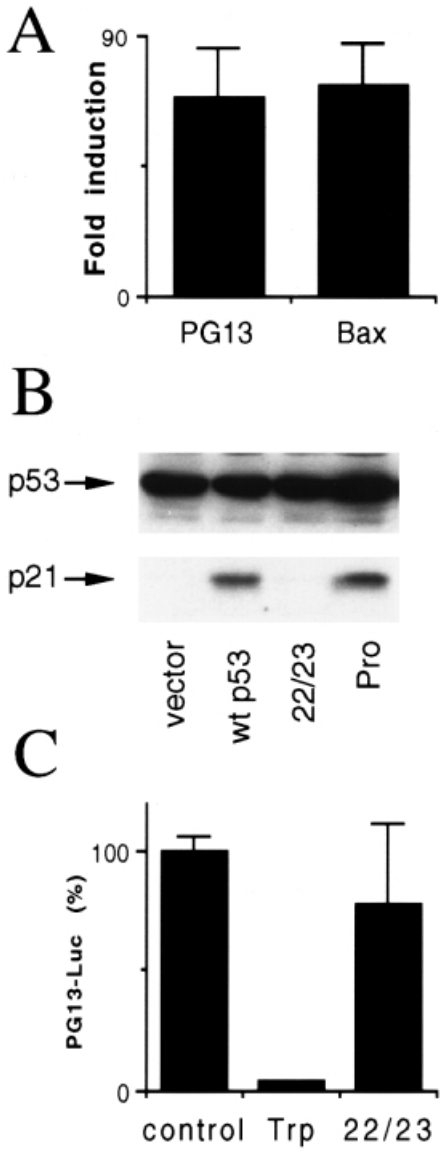

Fig. 6. Exogenous wild-type p53 is dominant over mutant p53. (**A**) Transactivation of PG13-Luc and Bax-Luc following transfection with wild-type p53 in SKBr3 cells harboring endogenous mutant p53. (**B**) SKBr3 cells were transfected with the indicated p53-expressing constructs and immunoblotting for p53 and p21 was performed as described in Materials and methods. (**C**) p53(22/23) is unable to transrepress a PG13-Luc construct in MCF-7 cells. Cells were co-transfected with PG13-Luc and either a p53-expressing construct or an empty vector (control).

a wild-type p53 allele (46). Furthermore, as in the parental cells, basal levels of p53 proteins were low in heterozygous clones (Figure 7B). Following DNA damage (+ adriamycin) induction of p53 was observed in both heterozygous clones and parental cells. In contrast, the PTX22 clone lacking a wild-type p53 allele expressed high basal levels of mutant p53 unresponsive to DNA damage (Figure 7B). We conclude that endogenous mutant p53 is not stable in the presence of a second p53 allele and therefore does not inhibit wild-type p53 activity.

Discussion

Wild-type p53 is a sequence-specific DNA-binding protein and transcription factor. In addition, p53 can repress transcription from several viral and cellular promoters, a property designated transrepression (18,19,47,48). This effect does not require sequence-specific DNA (22,42,49). For example, p53 represses the activity of HIF-1. Higher levels of p53 are required to inhibit HIF-1-stimulated transcription than to stimulate p53-dependent transcription (22,50,51).When highly overexpressed, p53 inhibits transcription in a less selective manner.

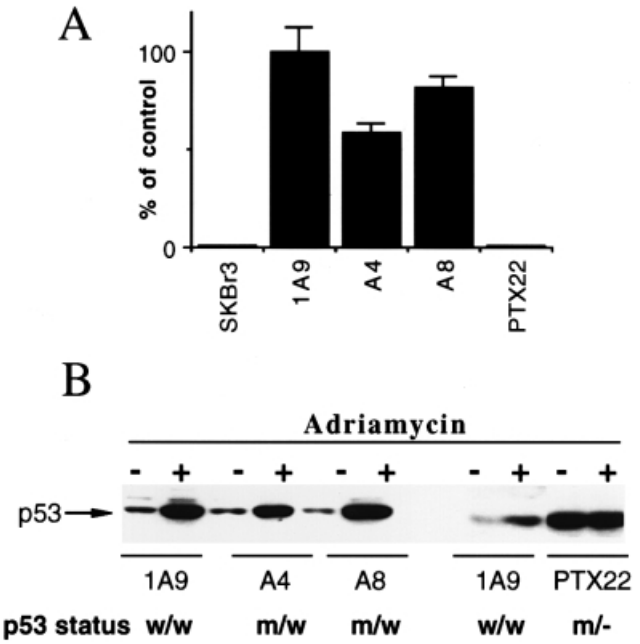

Fig. 7. Comparison of parental, natural heterozygous and homozygous p53 mutant cells. Parental A2780 (1A9) cells have two wild-type p53 alleles. Clones A4 and A8 have acquired an Asp245 mutation but also retain the second wild-type p53 allele (heterozygous clones). Clone PTX22 has acquired an Asp239 mutation and also lost the wild-type p53 allele (homozygous clone). (**A**) A2780 (1A9) cells, two heterozygous mutants (A4 and A8) and a homozygous mutant (PTX22) were transfected with PG3-Luc or PG13-Luc. SKBr3 (mutant p53) is shown for comparison. The ratio of PG13-Luc and pCMV-Luc was calculated to normalize the transfection efficiency. Results are presented as a percentage of transactivation in the parental cells, as the mean of three experiments. (**B**) A2780 (1A9) cells, two heterozygous mutants (A4 and A8) and a homozygous mutant (PTX22) were treated with 200 ng/ml adriamycin for 6 h (+) or left untreated (–). Immunoblotting for p53 was performed as described in Materials and methods.

In the present study we demonstrate that Arg175 mutants can repress HIF-1-stimulated transcription, albeit more weakly than wild-type p53. Both wild-type p53 and HIF-1 bind to and require p300, a transcriptional co-activator (40–44,52,53). It has been shown that, like wild-type p53, R248W and D281G mutants repress transcription of TIMP-3 (54).

We propose that, just as wild-type and mutant p53 can transrepress the activity of other transcription factors, transcriptionally inactive mutant p53s can repress wild-type p53-stimulated transcription, exerting what is commonly referred to as a dominant negative effect. Thus, competition for co-activator is not limited to the interaction of p53 (mutant or wild-type) with other transcription factors. A mutant p53 protein should be a very efficient competitor of endogenous wild-type p53 (manifested as a dominant negative phenotype). However, the dominant negative phenotype will only be manifested if the mutant p53 protein has lost its transactivating properties, such that it only competes for co-activator but cannot transactivate target promoters. Thus we show a perfect correlation between loss of transactivation function and the dominant negative effect. Importantly, Pro175 and Asn175 behaved like wild-type p53 on PG13 and p21 but failed to activate Bax-Luc. The proposed model predicts that Pro175 and Asn175 should selectively abrogate wild-type p53-stimulated Bax-Luc. We confirmed this prediction. Thus, if mutant p53s cannot transactivate a given promoter due to selective loss of transactivating ability they end up 'transrepressing' it (a dominant negative effect). In contrast, the 'conformational model' (in which mutant p53 drives wild-type p53 into the mutant conformation) predicts a dominant negative effect non-selective for any particular promoter. Our findings are in agreement with the recent demonstration that p53 mutants can possess selective dominant negative effects on apoptosis but not growth arrest due to differential repression of Bax and p21 (55). Furthermore, the fact that transactivation by mutant p53 may depend on cellular context may explain the observation that dominant negative effects are cell type dependent (56).

Dominant negative mutants are the most common found in human cancers (57). We suggest that this reflects loss of transcriptional activity of tumor-derived p53. This explains why selection for transcriptionally inactive p53 mutants yielded transdominant mutants, even though transdominance was not a requirement for mutant isolation (58). We conclude that a dominant negative effect is a consequence of loss of transactivating function. Finally, endogenous mutant p53 cannot efficiently repress endogenous wild-type p53 activity. As a result, loss of the second p53 allele is optimal since wild-type p53 function cannot be efficiently repressed; in the end, the easiest competitor is no competitor.

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