Oncogene (2000) 19, 3078–3085

www.nature.com/onc

Paclitaxel selects for mutant or pseudo-null p53 in drug resistance associated with tubulin mutations in human cancer

Paraskevi Giannakakou*[,1], George Poy[2], Zhirong Zhan[1], Turid Knutsen[1], Mikhail V Blagosklonny[1] and Tito Fojo[1]

[1]*Medicine Branch, National Cancer Institute, NIH, Bethesda, Maryland, MD 20892, USA;* [2]*Genetics and Biochemistry Branch, National Institute of Diabetes, Digestive and Kidney Diseases, NIH, Bethesda, Maryland, MD 20892, USA*

The efficacy of anticancer therapy is limited by the development of drug resistance. While the role of p53 in the intrinsic sensitivity of human cancer cells to paclitaxel (PTX) remains controversial, its role in acquired paclitaxel resistance has never been addressed. In this study we examined the p53 status of three paclitaxel selected human ovarian carcinoma sublines, resistant to paclitaxel due to acquired β-tubulin mutations which impair paclitaxel's interaction with tubulin. In contrast to parental cells which have wt p53, in all PTX-resistant sublines p53 was functionally inactive. Two of the resistant sublines expressed high levels of transcriptionally inactive p53 protein, each with a distinct point mutation in codons 236 and 239 of the DNA binding domain. The third subline presented a novel p53 pseudo-null phenotype as a result of markedly decreased wt p53 mRNA expression. Introduction of ectopic wt p53 had no effect on PTX sensitivity in both parental and resistant cells, while it induced $p21^{WAF1/CIP1}$, demonstrating an intact p53 pathway. While PTX resistance is primarily conferred by the tubulin mutations, the loss of functional p53 observed in all clones, suggests that this loss may facilitate the development of resistance potentially by providing a clonal advantage which promotes the isolation of paclitaxel resistant cells. *Oncogene* (2000) **19,** 3078–3085.

Keywords: paclitaxel; p53; tubulin; drug resistance; human cancer

Introduction

The p53 tumor suppressor protein is involved in cellular response to DNA damage (Levine, 1997). Accumulation of wild-type (wt) p53 following DNA damage (Kastan *et al*., 1991) results in induction of $p21^{WAF1/CIP1}$ (el-Deiry *et al*., 1993) and Bax (Miyashita and Reed, 1995) leading to growth arrest or apoptosis (Lowe *et al*., 1994). Mutations in p53, are the most common genetic abnormalities in human cancer (Nigro *et al*., 1989). Since p53 is also implicated in the mitotic checkpoint (Cross *et al*., 1995; Fukasawa *et al*., 1996), one can expect that loss of p53 function may affect the sensitivity to paclitaxel, a microtubule active drug. However, conflicting results have been reported regarding the role of p53 as a determinant of cell sensitivity to paclitaxel (Dumontet and Sikic, 1999). Although loss of p53 function increased the sensitivity of normal murine fibroblasts and some cancer cells to paclitaxel (Wahl *et al*., 1996; Zhang *et al*., 1998), p53 has no role in the intrinsic sensitivity of many human cancer cells (Debernardis *et al*., 1997; Fan *et al*., 1998; Weinstein *et al*., 1997; Gan *et al*., 1996; Vasey *et al*., 1996). Similarly, a lack of correlation between sensitivity to paclitaxel and p53 status was found in the 60 cell lines of the NCI anticancer drug screen (O'Connor *et al*., 1997). While these studies address the role of p53 on a cell's intrinsic sensitivity to paclitaxel, the role of p53 on acquired paclitaxel resistance remains unexplored. Acquired resistance develops in cells previously exposed to a cytotoxic drug. This type of resistance is a frequent clinical problem, which limits the effectiveness of therapy. It is this type of resistance that is addressed herein.

In the present study we describe PTX resistant human ovarian carcinoma sublines, which harbor β-tubulin mutations (Giannakakou *et al*., 1997); in two sublines, mutant p53 proteins were identified, while in the third, p53 protein was undetectable/uninducible as a result of marked downregulation of p53 mRNA – effectively a non-functional wt p53. Although infection with an adenovirus expressing wt p53 did not alter paclitaxel sensitivity, the loss of p53 function in all resistant clones suggests that a nonfunctional p53 is advantageous in the development of paclitaxel resistance.

Results

1A9 paclitaxel resistant clones have nonfunctional mutant p53

Wt p53 protein has a rapid turnover and is expressed at low levels in most cells and tissues. DNA damage causes rapid accumulation of p53 protein leading to transcriptional activation of target genes, such as $p21^{WAF1/CIP1}$ (Blagosklonny, 1997; Levine, 1997). To evaluate p53 function in parental 1A9 cells and the PTX-resistant clones, cells were treated with the DNA damaging agents Mitomycin C or Adriamycin (Figure 1). The two PTX-resistant clones designated PTX10 and PTX22 were maintained in 15 ng/ml paclitaxel, but were cultured in drug-free medium for 5–7 days prior to these experiments. Parental 1A9 cells had low basal levels of both p53 and p21. In parental cells treatment with either Mitomycin C or Adriamycin, increased the levels of both p53 and its transcriptional target, p21,

*Correspondence: P Giannakakou
Received 29 September 1999; revised 23 March 2000; accepted 28 March 2000

demonstrating that p53 was functional. Paclitaxel treatment resulted in a small increase in p53 levels compared to untreated parental cells. When similar studies were performed with the PTX-resistant clones, PTX10 and PTX22, high basal levels of p53 unresponsive to Adriamycin or Mitomycin C were observed. In these cells p53 was transcriptionally inactive as demonstrated by the lack of p21 induction (Figure 1). These results suggested that p53 in 1A9 parental cells was wt, but in the PTX-resistant clones had acquired mutations. To investigate this, sequencing of p53 from all cell lines was performed. In agreement with the results presented in Figure 1, sequence analysis revealed wt p53 in parental cells, with 'hot spot' mutations at the DNA binding domain of p53, in the PTX10 and PTX22-resistant clones (Table 1). The p53 mutation in clone PTX10 (236: Tyr→Cys) has been described as a dominant negative mutation (Brachmann *et al*., 1996) a different mutation was found in clone PTX22 (239: Asn→Asp). The substitution in codon 239 was present as a single peak in the sequence analysis indicating expression of wt mRNA could not be detected; while the substitution in codon 236 was present as two overlapping peaks indicating expression of both wt and mutant mRNA (not shown). This finding is consistent with a dominant-negative phenotype for the 236 substitution (Brachmann *et al*., 1996).

To assess the role of p53 in paclitaxel resistance, we investigated the effects of exogenous wt p53 on parental and PTX-resistant cells. As shown in Figure 2, p21 was undetectable by immunohistochemical staining, in untreated parental and PTX-resistant cells (Figure 2a,d). In agreement with the immunoblot results in Figure 1, following Adriamycin treatment, p21 expression was easily detected in parental cells but not in the PTX-resistant PTX22 cells (Figure 2b *vs* e). In contrast, infection with an adenovirus expressing wt p53 (Adp-53) induced p21 in both paclitaxel-sensitive and paclitaxel-resistant cells (Figure 2c,f), demonstrating that the lack of p21 induction in PTX22 cells after Adriamycin was a result of a nonfunctional p53; and that function could be restored by expressing exogenous wt p53.

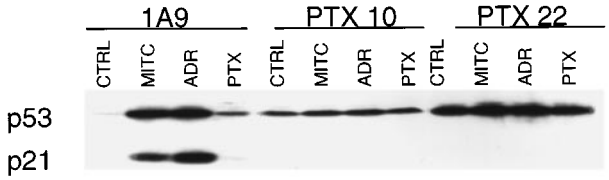

Figure 1 Characterization of p53 status in 1A9 paclitaxel resistant cells. 1A9 parental, PTX10, and PTX22 cells were treated with 10 mg/ml Mitomycin C (MITC), 400 ng/ml Adriamycin (ADR) or 1000 ng/ml paclitaxel (PTX) for 16 h. After lysis, p53 and p21 levels were detected by immunoblot as described in Materials and methods

Table 1 Mutation analysis of 1A9 PTX-resistant clones

Cell line	*β-tubulin (codon)*	*Codon change*	*p53 (codon)*	*Codon change*
PTX 10	**270**	TTT→GTT (Phe→Val)	**236 (+/−)**	TAC→TGC (Tyr→Cys)
PTX 15	**wt**	wt	**wt**	wt
PTX 22	**364**	GCA→ACA (Ala→Thr)	**239**	AAC→GAC (Asn→Asp)

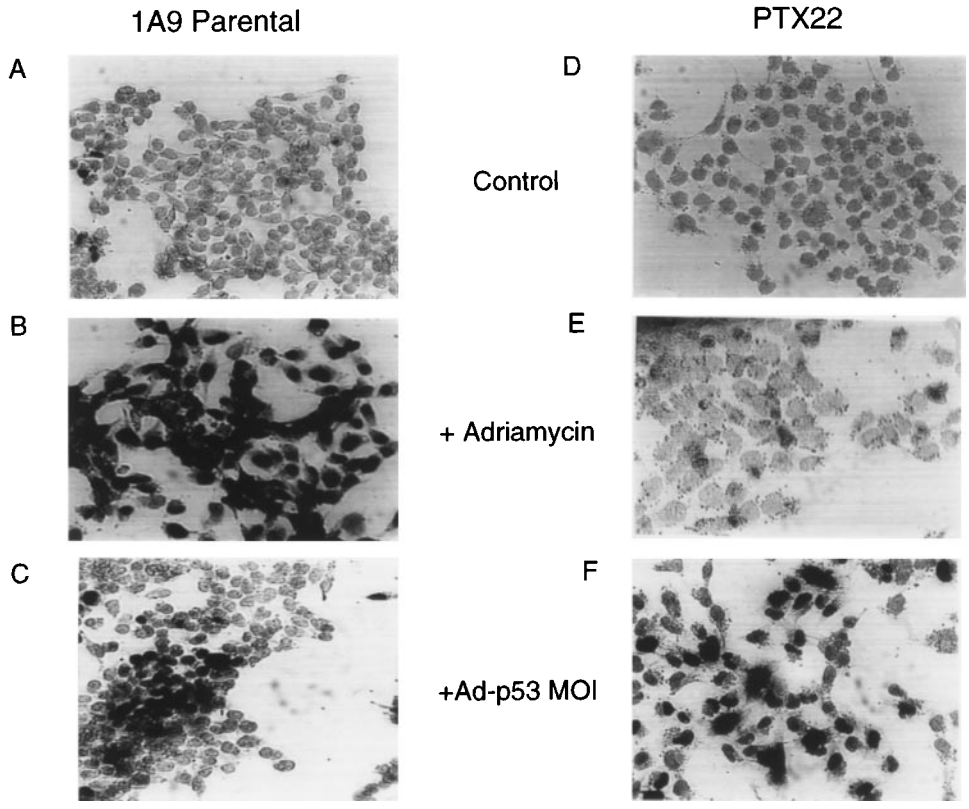

Figure 2 Induction of p21 by exogenous p53 in paclitaxel resistant cells. Immunohistochemical staining of 1A9 parental (**a–c**) and PTX 22 cells (**d–f**) with an antibody against human $p21^{WAF1/CIP1}$. (**a,d**): untreated control cells; (**b,e**): cells treated with 400 ng/ml Adriamycin for 24 h; and (**c,f**): cells infected with 20 MOI Ad-p53 for 24 h

Restoration of wt p53 did not increase the sensitivity to paclitaxel

To investigate the effect of exogenous wt p53 on paclitaxel sensitivity, 1A9 parental and PTX-resistant PTX10 and PTX22 cells were infected with Ad-p53. At high doses (MOI of 25 or greater), we observed that exogenous wt p53 caused greater growth inhibition of the resistant clones compared to parental 1A9 cells (Figure 3a). Thus, to avoid the confounding variable of changes in growth rate, which would impact sensitivity to paclitaxel, cells were infected with 3 and 10 MOI of Ad-p53. At these doses, despite evidence of adequate infection as demonstrated by induction of p21 (Figure 3b), neither parental nor resistant cells were sensitized to paclitaxel (Figure 3c). These results suggest that the selective survival advantage conferred by the loss of wt p53 was not a direct result of tolerance to paclitaxel cytotoxicity, but was likely mediated via another mechanism. It should be noted that infection with Ad-p53 did not further sensitize parental cells either.

Downregulation of p53 mRNA expression as another mechanism of loss of p53 function

To further investigate the loss of p53 function during the development of paclitaxel resistance we examined the p53 status of a third PTX-resistant clone designated, PTX15, which was an early isolate in the selection process and was maintained in 5 ng/ml paclitaxel. P53 and p21 proteins were undetectable in untreated PTX15 cells as well as after Adriamycin treatment (Figure 4a). Transfection with PG13-Luc, a p53-dependent plasmid, demonstrated high PG13-Luc expression in wt-p53 containing 1A9 cells, but not in either PTX22 or PTX15 cells (Figure 4b). The absence of p53 protein and p53 activity in PTX15 cells was consistent with p53-null status, which occurs when p53 is deleted or possesses a nonsense mutation. RT–PCR amplification of RNA from parental 1A9 cells and the three paclitaxel resistant clones using primers spanning exons 5–9 of p53, failed to identify a product in clone PTX15 (Figure 4c, upper panel), in agreement with the lack of p53 protein and compatible with a p53-null status. However, PCR amplification using DNA from PTX15 cells and primers spanning exons 6 and 7, resulted in a product (Figure 4c, lower panel) arguing against a deletion. Because some primer pairs perform better in PCR reactions and smaller fragments are easier to amplify, other primer pairs spanning smaller segments were used. These primers identified a product, using PTX15 mRNA, albeit at much lower levels (Figure 5). Note the differences in the amounts of input RNA in the PCR reactions (Figure 5). The resulting PCR products from PTX15 mRNA were shown to be wt in sequence (Table 1). Quantitative PCR showed that p53 mRNA levels in clone PTX15 were 150-fold less than parental 1A9 cells and 25–35-fold less than PTX10 and PTX22 cells, respectively, that had a mutant p53 (Figure 5). To investigate the mechanism responsible for the marked downregulation of p53 in PTX15 cells, FISH analysis using a p53 specific probe was performed in all cell lines. Like parental 1A9 cells, PTX10 and PTX22 clones contained two cytogenetically normal chromosomes 17, each with one copy of p53, located at chromosome band 17p13 (Figure 6). For PTX10 this was expected since sequence analysis revealed expression of both wt and mutant p53 sequences. However, surprisingly, PTX22 cells which expressed only the mutant p53 allele at the RNA level, also

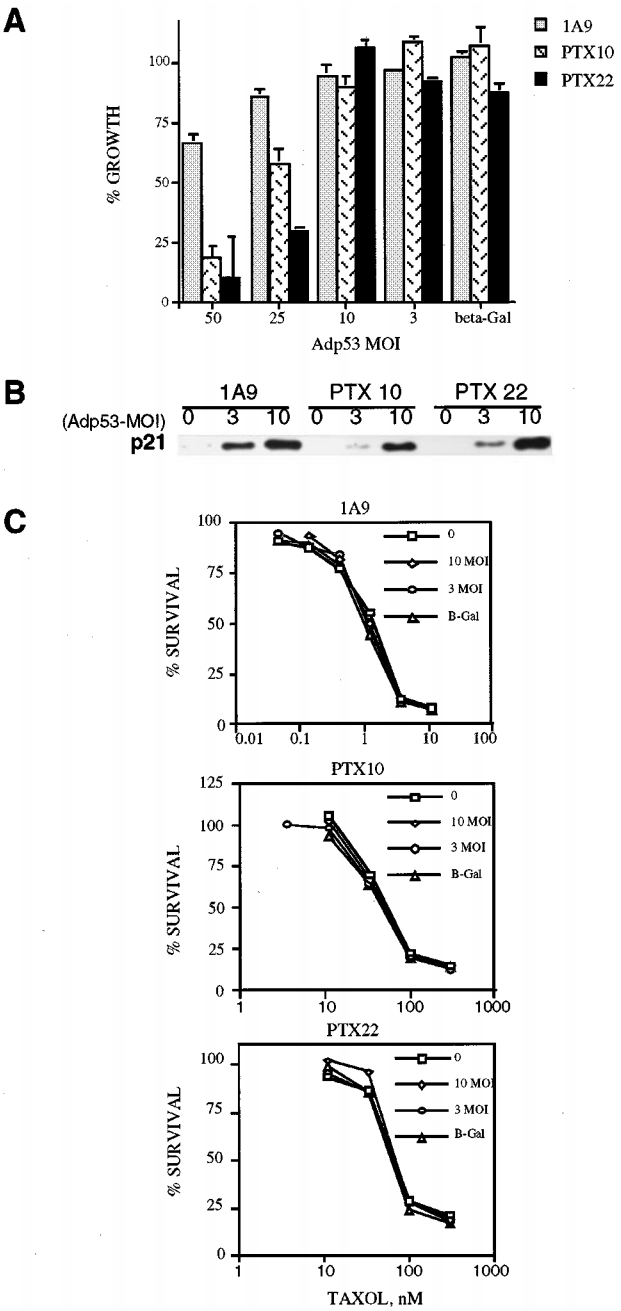

Figure 3 Expression of exogenous wt p53 does not alter sensitivity to paclitaxel in 1A9 and 1A9PTX cells. 1A9 parental, PTX10, and PTX22 cells were plated in 96 well plates and infected with increasing titers of Ad-p53, from 3–50 MOI, or with 50 MOI of Ad-LacZ. (**a**) Bar graph representation of the per cent growth of all three cell lines, 3 days post infection with p53 adenovirus shows growth inhibition in cells infected with 25 or 50 MOI. For each cell line, non-infected control cells were assigned a value of 100%. (**b**) Immunoblot demonstrating p21 expression in cells infected with 3 or 10 MOI Ad-p53. (**c**) Paclitaxel was added to 1A9, PTX10 and PTX22 cells 1 h post-infection with 3 or 10 MOI of Ad-p53 or 10 MOI of Ad-LacZ. The surviving fraction was determined 3 days later as described in Materials and methods

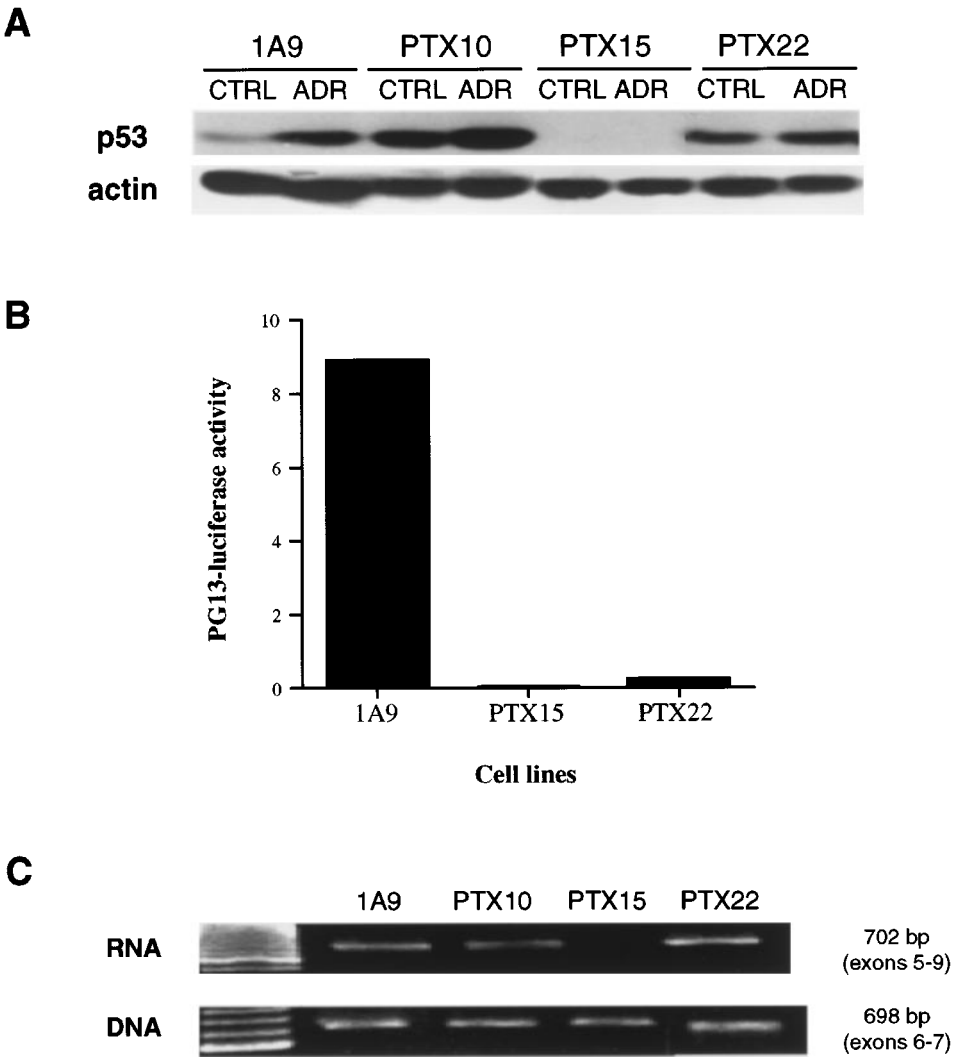

Figure 4 1A9PTX15 cells have undetectable p53 protein or mRNA levels. (**a**) Immunoblot analysis of p53 in untreated (CTRL) cells or cells treated with 400 ng/ml Adriamycin (ADR) for 16 h, is shown for 1A9, PTX10, PTX15 and PTX22 cell lines. The same blot reprobed with actin is shown below the p53 immunoblot for comparison. (**b**) Wild-type p53 functional activity in 1A9, PTX22 and PTX15 cells. PG13-luciferase activity was normalized to activity obtained with PGL2-control plasmid. (**c**) PCR amplification of p53 in 1A9, PTX10, PTX15 and PTX22 cells. Using RNA as a template and primers spanning exons 5–9 (upper panel) no product was observed in PTX15 cells. However, with DNA as a template and primers spanning exons 6–7 (lower panel), a product was detected in PTX15 cells

had two copies of p53 suggesting that the wt allele was silenced. The presence of a wt and mutant allele was confirmed by p53 sequencing using PTX22 DNA as a template. This revealed two overlapping peaks indicating that both wt and mutant alleles were present at the DNA level, even though only the mutant allele is expressed at the RNA level. PTX15 cells had one cytogenetically normal chromosome 17 with p53 and one translocation, t(17;?)(q10;?): this translocation contained the 17q arm but the 17p arm was deleted and there was no p53; the cell line PTX15, therefore, had only one copy of the p53 gene (Figure 6, arrow points to the chromosome 17p deletion). As demonstrated by the quantitative PCR results the expression of the remaining wt p53 allele is 150-fold downregulated in PTX15 compared to parental cells.

Taken together these results suggest that during the development of paclitaxel resistance, p53 became nonfunctional either as a result of mutation or marked downregulation of mRNA levels.

Discussion

Paclitaxel is effective against a wide variety of solid tumors, however, development of acquired drug resistance following chemotherapy occurs frequently in patients. A recently identified mechanism of PTX resistance is acquired point mutations in tubulin resulting in abrogation of tubulin/paclitaxel interactions (Giannakakou *et al*., 1997). Moreover, in a recent study of patients with non-small-cell lung cancer, the presence of β-tubulin mutations was negatively correlated with the response to paclitaxel (Monzo, *et al*., 1999). However, the p53 status was not evaluated in these patients, and we hope the present study will stimulate such clinical investigation. In this study we used PTX-resistant human ovarian carcinoma clones previously shown to harbor β-tubulin mutations (Giannakakou *et al*., 1997) as a model to investigate the role of p53 in the development of resistance. While DNA damaging anticancer agents are mutagenic, agents such as paclitaxel are not thought to cause

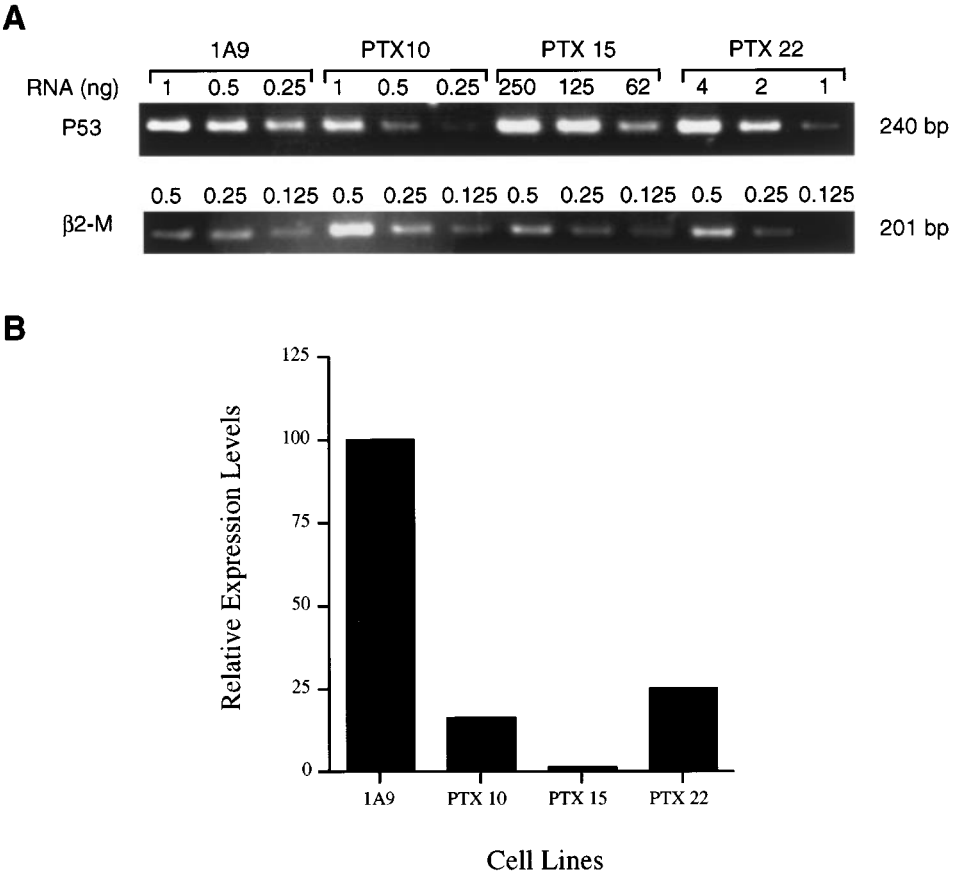

Figure 5 Downregulation of p53 mRNA as a mechanism of pseudonull status in 1A9PTX15 cells. (**a**) Quantitative RT–PCR amplification of a shorter fragment of p53 coding sequence, using primers spanning exons 6–8, and β2-microglobulin (β2-M) was performed in parental and PTX-resistant cells as described in Materials and methods. (**b**) Bar graph representation of the results displayed in (**a**). P53 expression levels are normalized to β2-microglobulin levels in each cell line. P53 expression levels in the 1A9 parental cells are arbitrarily assigned a value of 100. Relative to the 1A9 parental levels, the values of p53 expression levels for the PTX-resistant cell lines are: PTX10: 17; PTX15: 0.67; and PTX22: 25

point mutations, and it would be unlikely that paclitaxel would be directly responsible for point mutations. However, we demonstrate that all selected PTX-resistant cell lines exhibited non-functional p53 as a result of either acquired mutations or marked downregulation of mRNA expression.

Although the lack of PTX interaction with tubulin is the predominant cause of PTX resistance in the PTX10 and PTX22 ovarian cancer sublines, we could not exclude that a non functional p53 also contributed to the resistant phenotype. However, expression of wt p53 did not increase paclitaxel sensitivity in parental cells nor did it overcome the acquired PTX resistance that developed in the three resistant clones. While these results agree with previous observations in A2780 human ovarian carcinoma cells transfected with a dominant negative mutant p53 which acquired resistance to DNA damaging drugs but not paclitaxel (Vasey *et al*., 1996), we nevertheless had to reconcile the apparently contradictory observations that: (i) the cell lines isolated with paclitaxel all possess a non-functional p53; but (ii) restoration of a wild-type p53 status did not overcome the acquired paclitaxel resistance.

Conventional thinking about the selection of drug resistant sublines dictates that a change found after drug selection likely directly contributes to the drug resistance phenotype. However, in the present study, restoration of wild-type p53 did not restore the sensitivity. But why would all the resistant cells posses a mutant or nonfunctional p53? Neither long-term cultivation of parental cells nor clonal selection in the absence of paclitaxel (1A9 is a single cell clone of A2780 cells) changed wt p53 status, indicating that wt p53 status is preferable for parental cells.

Since paclitaxel by itself can not be responsible for the point mutations observed in the tubulin or in the p53, it is likely that paclitaxel selected cells with pre-existing p53 mutations. The selection is likely to have occurred because the process of isolation of mutant cells we used is a 'clonal selection' and it has been clearly demonstrated in the literature that cells with mutant p53 have greater cloning efficiency as evidenced by the formation of more tumors with shorter latency period, increasing clonogenicity and enhanced metastatic potential (Lowe *et al*., 1994; Hsiao *et al*., 1994a,b). Furthermore, once isolated, cells possessing a non-functional p53 likely had an additional advantage: the increased genomic instability seen in cells with mutant p53 (Hartwell, 1992; Havre *et al*., 1995; Liu *et al*., 1996) would likely accelerate acquisition of additional mutations. For example in our models, the two cell lines with acquired p53 mutations (PTX10 and PTX22) also acquired β-tubulin mutations (Giannakakou *et al*., 1997). In addition, selection of 1A9 cells with Epothilone A, an antitubulin natural product with

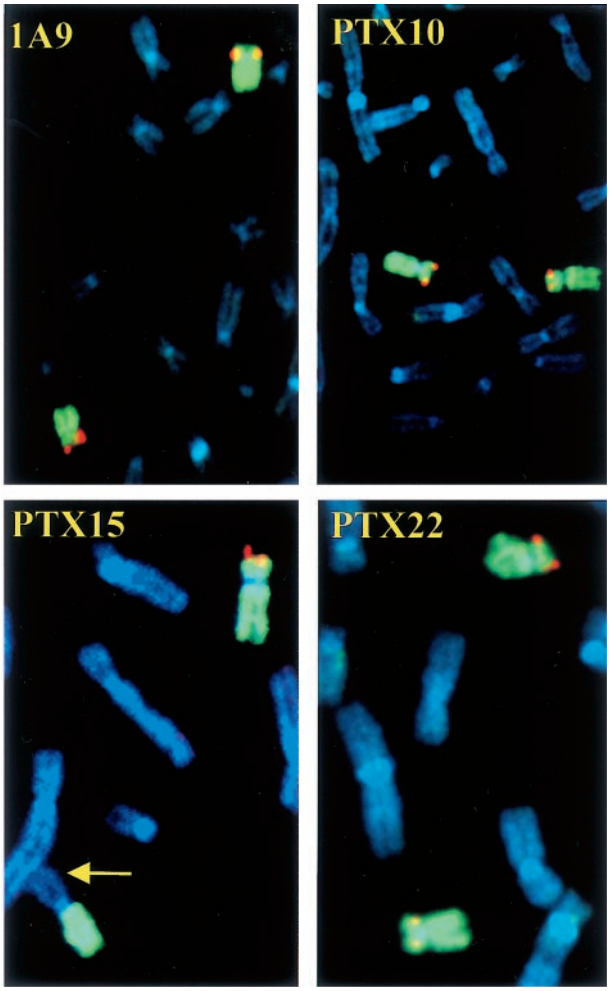

Figure 6 Fish analysis for p53 in 1A9, PTX10, PTX15 and PTX22 cells. Fish analysis was performed in parental 1A9 cells and the three selected PTX-resistant clones. A whole chromosome paint kit was used for chromosome 17 (WCP 17, green), and a locus specific probe p53 (red). DNA was counterstained with DAPI (blue). Arrow points to the chromosome 17p deletion in 1A9/PTX15 cells

a mechanism of action similar to PTX, yielded Epothilone-resistant clones that also had acquired p53 (not shown) and β-tubulin mutations (Giannakakou *et al*., 2000).

The PTX15 clone presented a novel mechanism which resulted in a p53 pseudo-null status. Sequence analysis demonstrated p53 to be wt in this cell line, but mRNA and protein levels were markedly reduced, effectively achieving the same effect as the p53 mutations – a non-functional status. By FISH analysis, PTX15 cells were shown to have a deletion at chromosome 17p that normally carries p53. This finding is consistent with the increased genomic instability observed in most cancers which results in losses and gains of whole chromosomes or large portions thereof (Lengauer *et al*., 1998). However, this can not fully explain the 150-fold downregulation of p53 mRNA expression. Instead it is clear that the downregulation is a result of reduced expression of the remaining allele. The hypothesis that the reduced expression is an acquired phenotype is supported by the results in PTX10 cells in which both the wt and mutant alleles are expressed at comparable levels, indicating that both alleles were transcribed in the parental cells from which the resistant sublines were derived. The frequency with which this downregulation may occur remains to be determined, although we recently described a similar observation in an anaplastic thyroid cancer cell line (Blagosklonny *et al*., 1998). In addition, similar downregulation appears to have occurred independently in the PTX22 clone which appears at the RNA level to be homozygous for mutant p53, but is in fact, heterozygous since: (a) FISH analysis revealed two normal chromosomes 17 carrying one copy of p53 in each and (b) DNA sequencing revealed the presence of both wt and mutant p53 at the DNA level. Silencing of p53 may predispose such clones to accumulate tubulin mutations. However, proving this will require measurements of the rate of acquired mutations in cells, which start out with mutated or p53-null status versus wild-type p53 cells. Detailed studies support the hypothesis that loss of p53 function leads to genetic instability allowing cells to accumulate mutations in many genes (Lengauer *et al*., 1998; Cahill *et al*., 1999). An alternate but non-mutually exclusive explanation would be that a mutant or p53-null status could provide a clonal advantage at particular stages of drug selection. One could also envision reduced p53 levels as an intermediate step which establishes a 'mutation permissive' p53 pseudo-null state, and this in turn leads to a 'fixed phenotype' with an acquired p53 mutation. Once the p53 mutation is acquired, cells do not need to control p53 expression, which can increase again.

In summary, the present study demonstrates that paclitaxel selects cells that harbor p53 mutations or are 'functionally' p53 mutant. The latter was achieved by a novel mechanism of reduced p53 mRNA expression. In the initial selection the cells with non-functional p53 likely had a clonal advantage. Beyond this, since loss of p53 function leads to genomic instability (Hartwell, 1992), cells with a downregulated wt p53 may have had much better chance of acquiring mutations that would confer resistance to the selecting agent. Acquisition of mutations in tubulin, made this process irreversible; thus the restoration of wt p53 did not return cells to more sensitive state.

Materials and methods

Cell lines and culture conditions

The 1A9 cell line is a clone of the human ovarian carcinoma cell line, A2780. The three PTX resistant sublines, PTX10, PTX15 and PTX22, were initially isolated as individual clones in a single step selection, by exposing 1A9 cells to 5 ng/ml PTX in the presence of 5 μg/ml verapamil, a Pgp antagonist. The three cell lines exhibit a non-MDR1 resistant phenotype and impaired PTX interaction with tubulin. After an initial expansion in 5 ng/ml PTX, PTX in the media was gradually increased to 15 ng/ml for clones PTX10 and PTX22; while clone PTX15 is an early isolate maintained in 5 ng/ml PTX. The cells grow in RPMI medium containing 10% FBS and were maintained in 15 or 5 ng/ml PTX and 5 μg/ml verapamil continuously. Drug was removed for 5–7 days prior to an experiment.

Immunochemistry

Immunochemistry was performed as previously described (Blagosklonny and el-Deiry, 1996). Nuclear staining was developed with the chromogen DAB (Sigma).

Immunoblot analysis

Twenty hours following infection of tumor cells or drug treatment, total cellular proteins were harvested and immunoblotted for $p21^{WAF1/CIP1}$ and p53, as previously described (Blagosklonny and el-Deiry, 1996).

Adenovirus infections

The replication deficient adenoviruses Ad-LacZ (β-galactosidase expressing) and Ad-p53 (wt p53-expressing) were generous gifts from Dr B Vogelstein. Titers were determined by plaque formation following infection of UP293 cells. The multiplicity of infection (MOI) was defined as the ratio of plaque forming units (PFU) divided by the number of cells. X-Galactosidase staining of Ad-LacZ infected cells was performed 1 day following infection as previously described (Blagosklonny and el-Deiry, 1996).

Cytotoxicity assay

Cytotoxicity assays were performed in 96-well plates using the Sulforhodamine B as previously described (Skehan *et al*., 1990). One thousand cells were seeded in each well in 100 μl of growth media. Twenty four hours later, adenovirus infection was performed by adding 50 μl of medium containing a defined amount of Ad-p53 or Ad-LacZ. Cytotoxic agents were added in 50 μl of medium 1 h after adenovirus infections, and cells were incubated for an additional 3 days. Untreated control cells were assigned a value of 100%, and the IC_{50} was defined as the dose of drug required to inhibit cell growth by 50%.

Luciferase assay

The p53-dependent PG-13-luciferase construct was obtained from Dr B Vogelstein. The PGL2 control plasmid driven by SV40 promoter and enhancer sequences, was purchased from Promega Corp. (Madison, WI, USA). One μg of either plasmid was transfected into 1A9, PTX15 and PTX22 cells using lipofectamine according to the manufacturer's instructions (Life Technologies, Inc.). Luciferase activity was measured in cell extracts 16 h after transfection using luciferase assay reagent (Promega). PG13-luciferase activity was normalized to the activity obtained with PGL2-control plasmid in each cell line tested.

Fluorescent in situ *hybridization*

Chromosome preparations of the cell lines 1A9, PTX10, PTX22, PTX15 were prepared according to previously described cytogenetics techniques. FISH was performed using direct labeled probes (Vysis, Downers Grove, IL, USA): a whole chromosome paint (WCP 17, Spectrum Green) kit for chromosome 17 (Cat. # 32-122017), and a locus specific probe for p53 (LSI p53 Spectrum Orange, cat. # 32-190008). All hybridizations were done overnight and all slides counterstained with 4′,6-diamidino-2-phenylindole (DAPI). The preparations were observed and analysed on a Leica DMRXA microscope equipped with Leica FISH image analysis system.

PCR amplification and sequence analysis of p53

Quantitative RT–PCR amplification and sequence analysis of p53 using RNA from 1A9 parental cells and the clones PTX10 and PTX22 were performed as previously described (Blagosklonny *et al*., 1998). Using RNA from PTX15 cells, a PCR product was not obtained, using primers previously described even after 45 cycles of amplification. Therefore, for PTX15 RNA, quantitative RT–PCR and sequencing were performed using primers that gave smaller PCR products, allowing amplification and sequencing of p53 as previously described (Murphy *et al*., 1990). The following primer pairs were used: a. *5′ UTR-Exon 5*: $5':^{-48}$GACACGGTTCCCTGGATTG^{-30}; $3':^{501}$CTGTGACTGCTTGTAGATGG^{483}, b. *Exon 4-Exon 10:* $5':^{339}$TTCTTGCATTCTGGGACAGCC^{357}; 3′:1043GCCTCATTCAGCTCTCGGAAC^{1022}, c. *Exon 9-3′ UTR:* $5':^{943}$CTCTCCCCAGCCAAAGAAG^{962}; $3':^{1760}$GAATTCAACAGTGAGGGACA^{1741}, d. *Exon 6-Exon 7:* $5':^{569}$CCTCCTCAGCATCTTATCC^{586}; $3':^{704}$TTGTAGTGGATGGTGGTACAG^{684}, when DNA is used for the amplification and e. *Exon 6-Exon 8:* $5':^{569}$CCTCCTCAGCATCTTATCC^{586}, $3':^{802}$TTCCGTCCCAGTAGATTACC^{784} for the quantitative RT–PCR.

Abbreviations

The abbreviations used are: wt, wild-type; PTX, paclitaxel; MOI, multiplicity of infection; PFU, plaque forming units; Ad-p53, adenovirus expressing wt p53; Ad-LacZ, adenovirus expressing β-galactosidase.

References

Blagosklonny MV (1997). *Oncogene,* **15,** 1889–1893.
Blagosklonny M V and el-Deiry WS. (1996). *Int. J. Cancer,* **67,** 386–392.
Blagosklonny MV, Giannakakou P, Wojtowicz M, Romanova LY, Ain KB, Bates SE and Fojo T. (1998). *J. Clin. Endocrinol. Metab.,* **83,** 2516–2522.
Brachmann RK, Vidal M and Boeke JD. (1996). *Proc. Natl. Acad. Sci. USA,* **93,** 4091–4095.
Cahill DP, Kinzler KW, Vogelstein B and Lengauer C. (1999). *Trends Cell Biol.,* **9,** 57–60.
Cross SM, Sanchez CA, Morgan CA, Schimke MK, Ramel S, Idzerda RL, Raskind WH and Reid BJ. (1995). *Science,* **267,** 1353–1356.
Debernardis D, Sire EG, De Feudis P, Vikhanskaya F, Valenti M, Russo P, Parodi S, D'Incalci M and Broggini M. (1997). *Cancer Res.,* **57,** 870–874.
Dumontet C and Sikic BI. (1999). *J. Clin. Oncol.,* **17,** 1061–1070.
el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW and Vogelstein B. (1993). *Cell,* **75,** 817–825.
Fan S, Cherney B, Reinhold W, Rucker K and O'Connor PM. (1998). *Clin. Cancer Res.,* **4,** 1047–1054.
Fukasawa K, Choi T, Kuriyama R, Rulong S and Vande WG. (1996). *Science,* **271,** 1744–1747.
Gan Y, Wientjes MG, Schuller DE and Au JL. (1996). *Cancer Res.,* **56,** 2086–2093.
Giannakakou P, Sackett DL, Kang YK, Zhan Z, Buters JT, Fojo T and Poruchynsky MS. (1997). *J. Biol. Chem.,* **272,** 17118–17125.
Giannakakou P, Gussio R, Nogales E, Downing KH, Zaharevitz D, Bollbuck B, Poy G, Sackett D, Nicolaou KC and Fojo T. (2000). *Proc. Natl. Acad. Sci. USA,* **97,** 2910–2915
Hartwell L. (1992). *Cell,* **71,** 543–546.
Havre PA, Yuan J, Hedrick L, Cho KR and Glazer PM. (1995). *Cancer Res.,* **55,** 4420–4424.
Hsiao M, Low J, Dorn E, Ku D, Pattengale P, Yeargin J and Haas M. (1994a). *Am. J. Pathol.,* **145,** 702–714.
Hsiao MH, Yu AL, Yeargin J, Ku D and Haas M. (1994b). *Blood,* **83,** 2922–2930.

Kastan MB, Onyekwere O, Sidransky D, Vogelstein B and Craig RW. (1991). *Cancer Res.,* **51,** 6304–6311.

Lengauer C, Kinzler KW and Vogelstein B. (1998). *Nature,* **396,** 643–649.

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

Liu PK, Kraus E, Wu TA, Strong LC and Tainsky MA. (1996). *Oncogene,* **12,** 2267–2278.

Lowe SW, Bodis S, McClatchey A, Remington L, Ruley HE, Fisher DE, Housman DE and Jacks T. (1994). *Science,* **266,** 807–810.

Miyashita T and Reed JC. (1995). *Cell,* **80,** 293–299.

Monzo M, Rosell R, Sanchez JJ, Lee JS, O'Brate A, Gonzalez-Larriba JL, Alberola V, Lorenzo JC, Nunez L, Ro JY and Martin C. (1999). *J. Clin. Oncol.,* **17,** 1786–1793.

Murphy LD, Herzog CE, Rudick JB, Fojo AT and Bates SE. (1990). *Biochemistry,* **29,** 10351–10356.

Nigro JM, Baker SJ, Preisinger AC, Jessup JM, Hostetter R, Cleary K, Bigner SH, Davidson N, Baylin S and Devilee P. (1989). *Nature,* **342,** 705–708.

O'Connor PM, Jackman J, Bae I, Myers TG, Fan S, Mutoh M, Scudiero DA, Monks A, Sausville EA, Weinstein JN, Friend S, Fornace AJJ and Kohn KW. (1997). *Cancer Res.,* **57,** 4285–4300.

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S and Boyd MR. (1990). *J. Natl. Cancer Inst.,* **82,** 1107–1112.

Vasey PA, Jones NA, Jenkins S, Dive C and Brown R. (1996). *Mol. Pharmacol.,* **50,** 1536–1540.

Wahl, AF, Donaldson, KL, Fairchild, C, Lee, FY, Foster, SA, Demers, GW and Galloway, DA. (1996). *Nat. Med.,* **2,** 72–79.

Weinstein JN, Myers TG, O'Connor PM, Friend SH, Fornace AJJ, Kohn KW, Fojo T, Bates SE, Rubinstein LV, Anderson NL, Buolamwini JK, van Osdol WW, Monks AP, Scudiero DA, Sausville EA, Zaharevitz DW, Bunow B, Viswanadhan VN, Johnson GS, Wittes RE and Paull KD. (1997). *Science,* **275,** 343–349.

Zhang CC, Yang JM, White E, Murphy M, Levine A and Hait WN. (1998). *Oncogene,* **16,** 1617–1624.