Increasing P53 Protein Sensitizes Non-Small Cell Lung Cancer to Paclitaxel and Cisplatin *In Vitro*

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Abstract. *Aim: To determine whether increasing p53 protein levels confers enhanced chemosensitivity in non-small cell lung cancer (NSCLC). Materials and Methods: Three NSCLC cell lines, with different endogenous p53 expression, were transfected with wild-type p53 (wt-p53) or CD-1 (truncated wt-p53) genes. Cells were subsequently treated with cisplatin (CDDP) or paclitaxel (PAX). Cell viability was measured using Alamar Blue Assay. Results: Cells transfected with CD-1 expressed 13-38% higher levels of p53 protein compared to cells transfected with the wt-p53 gene, despite their baseline endogenous levels. CD-1-transfected cells also had higher cell death when treated with CDDP ($p<0.05$) or PAX, exhibiting 30-60% higher death rates than cells transfected with the wt-p53 gene and 130-160% higher than untransfected cells. A significant positive correlation between p53 protein concentration and cytotoxic response was demonstrated (R^2 for CDDP=0.823; R^2 for PAX=0.909; $p<0.001$). Conclusion: Increasing intracellular p53 protein concentrations can augment the effect of CDDP and PAX in NSCLC, despite the baseline level of p53 protein expression.*

Induction of the *p53* tumor suppressor gene results in pleiotropic effects upon the cell cycle, including cell cycle control, DNA synthesis and repair, growth factor regulation, and programmed cell death. *P53* gene mutations are among the most frequent molecular events in carcinogenesis, found in up to 60% of cases of non-small cell lung cancer (NSCLC) and 90% of small cell lung cancer (1, 2). Loss of *p53* function results in unregulated proliferation of damaged cells, and chemoresistance (3). The high prevalence of *p53* mutations (1), their detection early in the course of tumorigenesis (2, 4), and their correlation with a poor prognosis suggest that loss of normal *p53* function is an important step in NSCLC oncogenesis and tumor progression (5, 6).

In cells with *p53* mutations, restoration of wild type *p53* (wt-*p53*) function by gene transfection reinstates normal apoptosis (7, 8), resulting in increased cytotoxicity *in vitro* (9) and tumor regression *in vivo* (10-11). Restoration or endogenous expression of wt-*p53* also influences reliable cancer response to chemo- and radiation therapy in solid tumors including lung cancer (3, 12-14).

Response of tumors to the first-line agent cisplatin (CDDP) is mediated through a *p53*-dependent apoptotic pathway (3, 15-18). Aberrant *p53* expression is significantly associated with CDDP resistance in NSCLC (19). Several authors have also demonstrated the return of NSCLC sensitivity to CDDP when wt-*p53* status is reinstated by wt-*p53* gene transfection (3, 16, 20).

The relationship between *p53* status and NSCLC response to paclitaxel (PAX) is not completely understood. Studies performed on non-pulmonary solid tumors suggest that response to PAX may be independent of *p53* status (3, 17, 21-25). In contrast, other data have suggested that PAX sensitivity may be dependent on *p53* status, demonstrating increased G_1 cell arrest when lung cancer cells were transfected with wt-*p53* gene prior to treatment with taxols (26). Additive/ synergistic effects of PAX were seen when cancer of epithelial origin was transfected with wt-*p53* (27). However, few of these studies include lung cancer in their investigations. Reports that transfection of NSCLC cells with

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wt-*p53* results in enhanced sensitivity of NSCLC cells to chemotherapy (3, 16) and radiotherapy (11), resulting in human tumor regression in patients with advanced NSCLC, further emphasize the need for restoring *p53* function in this type of lung cancer.

The role of supra-physiologic levels of p53 protein, beyond those achieved with wt-*p53* transfection, on restoration of NSCLC apoptosis and therapeutic efficacy of anticancer agents is not understood. Some tumors are resistant to attempts at restoring wt-*p53* function, whereas others are susceptible to it (28, 29). Cells refractory to wt-*p53* transfection may have more extensive changes in their DNA profile (7), requiring more potent DNA activation modalities to overcome the effects of *p53* oncogenic mutants. A recent study investigating human NSCLC *in vitro* and *in vivo* suggested that an indirect increase in p53 protein expression by exogenous delivery of the tumor suppressor gene, *FUS1*, could enhance chemosensitivity to CDDP through the *p53*-dependent pathway (30). However, direct quantitative correlation of levels of p53 protein and CDDP response had not been studied. Inoue *et al*. (26) suggested that NSCLC cells with higher amounts of functional p53 protein were more sensitive to anticancer drugs, as compared with NSCLC cells expressing baseline endogenous quantities of p53 protein. This was found indirectly when A549 cells (NSCLC cell line with functional endogenous wt-*p53*, expressing endogenous p53 proteins) were more sensitive to combination treatment with anticancer drugs and adenoviral vector with wt-*p53* gene (Ad-wt-*p53*), resulting in overall supra-physiologic p53 protein levels, than were NSCLC cells with deleted or mutated *p53* genes (26).

To further elucidate the effect of supra-physiologic p53 protein expression on restoration of *p53*-dependent apoptosis and chemosensitivity, a non-viral vector was employed to transfect NSCLC cells with a truncated version of the wt-*p53* gene (31). This unique truncated *p53* gene, *CD-1*, lacks the C-terminal regulatory phosphorylation sites of the *p53* gene, thereby permitting unregulated protein expression resulting in higher cellular protein levels. Cell transfection with *CD-1* results in expression of a functional p53 protein at 1.5- to 2-fold higher levels and longer intracellular half life than those achieved with wt-*p53* transfection (31). In the current study, we hypothesized that transfection of NSCLC cells with *CD-1* would result in increased p53 protein expression and function compared to cells transfected with wt-*p53*, and that higher protein expression consequently enhances NSCLC chemosensitivity to CDDP and PAX.

Materials and Methods

Cell lines and cell culture. Three human NSCLC cell lines (A549, H358, and H1299) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The ATCC characterizes the cell lines by cytogenetic analysis, DNA profiling (short tandem repeats) or a combination of both to ensure the identity of each cell line. Upon purchase, cell lines were maintained in RPMI-1640 medium supplemented with gentamicin and 10% fetal bovine serum (FBS). All cells were grown at 37˚C in a humidified incubator with 5% CO_2. These cell lines were chosen because of their endogenous *p53* gene status: A549 cells have endogenous wt-*p53*, whereas H358 and H1299 cells carry mutated and deleted *p53* genes, respectively. The cells were stored at –80˚C until experiments were performed. Approximately 250,000 cells were thawed at 37˚C and incubated in 3.5 ml RPMI-1640. Cells were passaged twice, for a total of one to two weeks, before they were in log-phase growth and ready for the experimental assays described below. Cells were sub-cultured at 50-75% confluence.

Plasmid vectors. Recombinant plasmid vectors were obtained from Dr. Y. K. Fung (University of Southern California, Children's Hospital of Los Angeles, CA, USA). Two gene constructs, under the control of the cytomegalovirus (CMV) promoter/enhancer were used in this study: (i) pCMV-*p53*+ (wt-*p53*), and (ii) pCMV-*CD-1* (truncated p53 gene translates codons 1-366), a *p53* gene with truncated *C*-terminal regulatory domain (31). P53 protein resulting from *CD-1* (Figure 1) translation has previously been demonstrated to have conserved protein function (31).

PEI-DNA plasmid formulations. Polyethylenimine (PEI), a 25 kDa branched cationic polymer vector (Aldrich Chemicals, Milwaukee, WI, USA), was used to transport the DNA plasmid vectors into the NSCLC cells. PEI was chosen because the Authors' prior experience has shown it to have reliable experimental transfection and genetic expression (32, 33) compared with other viral and non-viral vectors. A PEI stock solution was prepared in 1X PBS at a concentration of 4.3 mg/ml (0.1M in nitrogen). Transfection solution was prepared by mixing 2.1 μl of PEI stock and 7 μg DNA (wt-*p53* or *CD-1*) in 0.5 ml H_2O. This was based on the Authors' experience with previously optimized and re-confirmed PEI nitrogen: plasmid DNA phosphate (N:P) charge ratios of 10:1 (32, 34). The solution was incubated at room temperature for 15 minutes. After ensuring that no DNA precipitate was present, 3.0 ml of RPMI-1640 with 10% FBS were added.

PEI-DNA transfection. Cells were seeded into 96-well plates at optimal densities for 24 hours: A549 at 1,000 cells/well, H1299 at 2,000 cells/well, and H358 at 3,000 cells/ well. Transfection solution (100 μl/well) prepared as described above was then added after removing media supernatant from the previous day. Cells were incubated at 37˚C for 24-48 hours in transfection solution to achieve sub-confluent growth in each well.

P53 ELISA of transfected cells. P53 protein expression of cells transfected with wt-*p53* and *CD-1* gene constructs (as above) was evaluated using a Human p53 ELISA kit (R&D Systems, Minneapolis, MN, USA). Cells were washed with PBS and lysed with radioimmunoprecipitation assay (RIPA) lysis buffer (20 mM Tris, 0.5 mM EDTA, 1% Nonidet P40, 0.1% SDS, 1 mM PMSF, 100 mM Na Orthovanadate, 0.5 TIU/ ml aprotinin) for 15 minutes on ice. Samples were centrifuged for 10 minutes at 10,000×*g*. Supernatant (100 μl) was used for ELISA assay after p53 protein concentrations were standardized at 280 nm absorbance.

P53 Western blot analysis of transfected cells. Whole cell protein extracts of *p53* and *CD-1*-transfected cells were analyzed by Western blot. Cells were lysed as above and protein quantified by Bradford

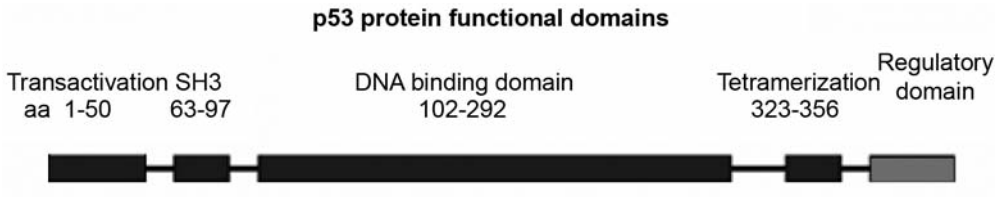

Figure 1. *P53 protein functional domains. CD-1 gene expresses all domains except the regulatory domain, responsible for inactivation of the p53 protein.*

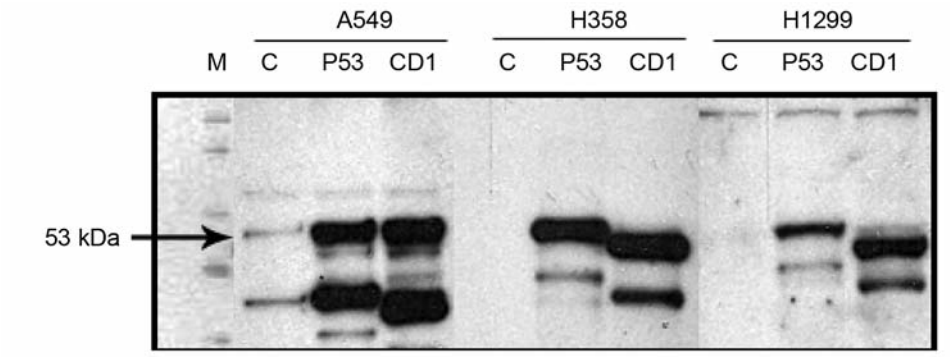

Figure 2. *Western blot of p53 protein in transfected NSCLC cells. Basal p53 protein (column labeled C) is seen in only A549 cells. Transfection with wt-p53 and CD-1 induced higher protein expression in all three cell lines, compared with control (C). Protein standard in kDA, marker (M).*

assay (Bio-Rad Laboratories, Hercules, CA, USA). Forty micrograms of protein from each sample were then separated by electrophoresis on a 10% NuPage Novex Bis Tris gel and electrically transferred to an Invitrolon™ PVDF 0.45 μm pore membrane using an XCell Surelock Mini-Gel system and XCell II blot module (Invitrogen, Carlsbad, CA, USA). Western blot was probed for p53 protein with polyclonal rabbit antibodies generated against the human whole p53 protein molecule (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA). Resulting immunoblots were analyzed using the Pierce ECL enhanced chemiluminescent system according to the manufacturer's recommendation. Band intensities, by digital pixel quantification, were used to estimate relative differences in p53 protein expression between *p53* and *CD-1* gene transfection. Digital pixel quantification was performed using the Un-Scan-It Graph™ Digitizing Software (Silk Scientific Inc., Orem, UT, USA).

Chemosensitivity assay. Cells were grown and transfected with wt-*p53* or *CD-1* as described above. When minimum 50% confluence of growth was reached, cells were incubated for 48 hours with CDDP (0.26-16.6 μM) or PAX (0.42-27 nM), with RPMI-1640 media as control (n=4 for each drug treatment). Drug cytotoxicity on exponentially growing cells was determined using the Alamar Blue Assay. When added to each well at 1/10 volume ratio, the Alamar Blue dye detects viable cells by reducing the ambient blue media to fluorescent red. The 96-well plates were assayed for fluorometric reduction at 530/30 nm excitation and 590/50 nm emission in the Cytofluor II plate reader (Applied Biosystems, Foster City, CA, USA). Samples were analyzed for chemical reduction (indicating cell viability) at 4 hours after the dye was added. Alamar Blue Assay was non-toxic to cells, allowing kinetic assays to be performed. Drug cytotoxicity, from CDDP or PAX, of transfected cells relative to untransfected (control) cells was calculated for both wt-*p53* and *CD-1*. Percentage enhancement of drug-induced cell death with *CD-1 versus* wt-*p53* transfection was determined as follows: [(cell cytotoxicity with CD-1+drug)/(cell cytotoxicity with wt-*p53*+drug)]×100=cytotoxic enhancement.

Data analysis. Student's *t*-test was used to compare differences in p53 protein expression between wt-*p53*-transfected and *CD-1*-transfected cells with significance set at $p<0.05$. A one-way ANOVA was used to determine significance between cytotoxic enhancements of *CD-1* and wt-*p53*-transfected cells treated with drug. Pearson correlation was used to determine the relationship between the increases in level of p53 protein expression and cytotoxic enhancement with each drug.

Results

P53 protein expression in transfected cells. Three wt-*p53* gene- and *CD-1* gene -transfected cell lines (A549, H358 and H1299) were compared with their untransfected counterparts. As expected, untransfected A549 cells expressed endogenous p53 protein, whereas H358 and H1299 did not, when probed for human wild-type p53 protein (Figure 2). In all 3 cell lines at least 100% increase in p53 protein expression was demonstrated when cells were transfected with wt-*p53*, by Western blot analysis. However, *CD-1* gene transfection into all three NSCLC cell lines, resulted in the highest p53 protein expression when compared with wt-*p53* transfection and untransfected controls. This was demonstrated by human p53 ELISA (Table I) and Western blot analysis (Figure 2). Using pixel comparison on Western blot (Image J, National Institutes of Health,

Table I. *P53 protein ELISA assay of transfected NSCLC cells.*

	Untransfected	Wt-*p53* transfected	*CD-1*-transfected	CD-1/wt-*p53* ratio
A549	0.2545	0.4345 (170%)	0.5865 (230%)	1.35
H358	0.2265	0.3525 (155%)	0.63 (278%)	1.79
H1299	0.1575	0.788 (500%)	1.2345 (783%)	1.57

Values are expressed in optical density (OD) units. Values in parentheses indicate % relative to the control. Data from p53 protein ELISA assay of NSCLC cells transfected with gene constructs in comparison with untransfected controls. All three NSCLC cell lines tested express higher p53 protein when transfected with wt-*p53* gene construct compared with *CD-1* gene construct.

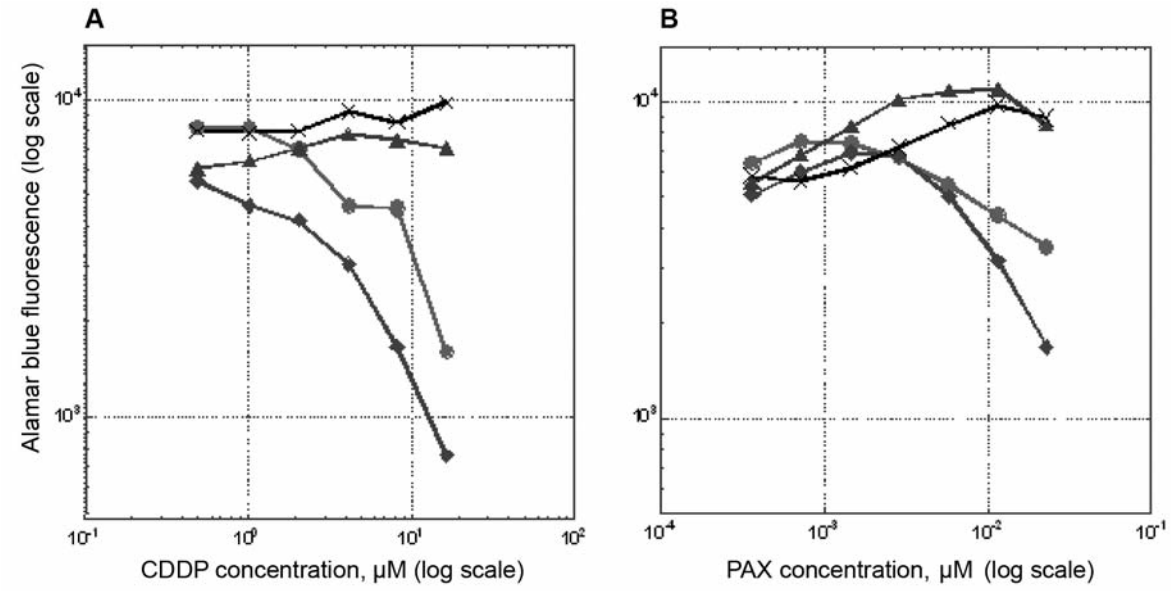

Figure 3. *H1299 cell viability expressed as fluorescence (log scale). Cells treated with drug alone (●), CD-1 gene transfection alone (▲), or treated with drug after CD-1 transfection (♦) are compared with untreated controls (×). Treatment with drug (A=CDDP, B=PAX) after CD-1 transfection results in the lowest cancer cell viability. Please note the difference in log concentrations of CDDP and PAX on the abscissa.*

Bethesda, MD, USA), a higher p53 protein expression in *CD-1*-transfected cells was demonstrated as follows: A549 had a 13% increase in p53 protein expression; H358 had a 26% increase; and H1299 had a 38% increase, when compared with wt-*p53*-transfected cells.

Cytotoxic enhancement with wt-p53 and CD-1 transfection compared to baseline. NSCLC cells treated with CDDP and PAX did result in cell cytotoxicity (Figure 3). After transfection with wt-*p53*, increased cell death was noted in response to CDDP (range 13-32%) and PAX (range 35-38%) treatment in all 3 cell lines (Figures 3 and 4). This increase in cytotoxicity was noted regardless of the baseline *p53* status of the cells. Cytotoxicity of CDDP and PAX was further enhanced by transfection with *CD-1* in all 3 cell lines (Figure 4). Notably, the increased cell death in response to CDDP, with *CD-1* compared to wt-*p53* transfection, was significantly higher in cells lacking endogenous p53 protein expression (H358 and H1299, $p<0.05$; A549, $p=0.054$; n=4) (Figure 4A). Overall p53 protein effect was greater in PAX treated cells for both wt-*p53* and *CD-1* transfection, when cells were treated with PAX at concentrations of 27 nM (Figure 4B). Statistical significance of *CD-1 versus* wt-*p53* transfection, in PAX treated cells, was reached only for H1299, $p=0.031$. Cytotoxic enhancement by *CD-1* compared with wt-*p53* effect was expressed as a ratio, CD-1:p53, in Figure 4. wt-*p53* and *CD-1* gene transfection alone, in the absence of either drug, had negligible effect on cell viability (Figure 3). As expected, treatment with drug alone had cytotoxic effects at appropriate concentrations (CDDP>2.1 μM; PAX>2.9 nM). An additive effect was seen with combination of *CD-1* transfection with CDDP treatment throughout the range of tested concentrations (Figure 3A). A similar effect was seen with PAX and *CD-1* combination at higher doses of PAX (Figure 3B). Similar effects were seen with wt-*p53* transfection prior to treatment with either drug (Data not shown).

Enhancement of p53 expression and increase in cytotoxicity with anticancer drugs. Both CDDP and PAX enhanced

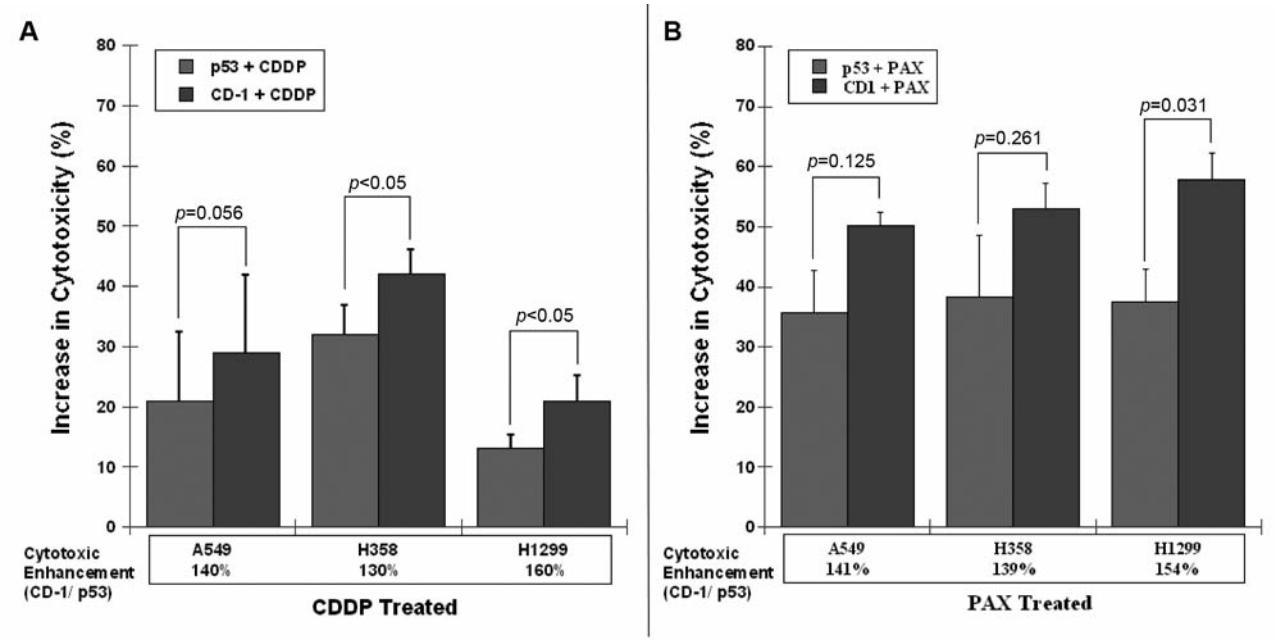

Figure 4. *CDDP (A) and PAX (B) cytotoxicity in wt-p53- and CD-1-transfected cells. Data are reported as increase in cytotoxicity relative to untransfected cells. Cytotoxic enhancement of CD-1 compared with wt-p53 is reported as cytotoxic enhancement ratio. A: CD-1 transfection resulted in a significant enhancement of cell death when cells were treated with a range of CDDP (0.26-16.6 μM), n=4. B: CD-1 transfection resulted in enhancement of cell death when cells were treated with PAX at 27 nM concentration. Significance noted in H1299 only, p<0.05, n=4.*

cytotoxicity in a protein-dependent manner in all three cell lines. Pearson correlation coefficient between p53 protein expression and enhancement of cytotoxicity was significant for both CDDP enhancement (R^2=0.823) and PAX enhancement (R^2=0.909) as seen in Figure 5.

Discussion

This study examined the effect of two different *p53* genes on the efficacy of CDDP and PAX cytotoxicity in human non-small cell lung cancers. An enhanced response of *in vitro* human NSCLC cells to both CDDP and PAX when first transfected with a wild-type *p53* gene vector was demonstrated. In addition it was found that that increasing the levels of functional p53 protein, beyond those previously achieved with wt-*p53* transfection, resulted in even higher chemosensitivity of cells treated with CDDP or PAX. This enhanced effect was demonstrated using a novel truncated version of wt-*p53*, previously theorized to express elevated levels of p53 protein (31), in comparison with traditional whole wt-*p53* gene used in other studies. While the increased susceptibility to CDDP and PAX was observed in all tested cell lines, regardless of their endogenous *p53* status, the effect approached significance in the cell lines (H358 and H1299) carrying *p53* mutations or deletions. A definite positive correlation was noted between the level of cellular p53 protein expression and cytotoxic enhancement from either CDDP or PAX.

Tumors with mutated *p53* genes are less sensitive to CDDP than those with wild-type *p53* genes expressing normal protein (3). Reinstating wild-type function restores chemosensitivity to CDDP (3, 10, 15-16). The sole importance of *p53* has been demonstrated when *in vivo* intra-tumoral injection of *p53* alone resulted in clinically apparent tumor regression (10). *P53* gene mutation status was recently correlated to chemoresistance and worse prognosis *in vivo* and *ex vivo* (35-36). The current results are in concordance with prior studies demonstrating a need for a functional wt-*p53* gene and protein for CDDP-induced apoptotic effect on lung cancer cells (3, 19, 36). However, this study also demonstrated an even greater drug effect by stimulating production of supra-physiologic quantities of p53 protein.

The role of wt-*p53* status in the effect of PAX against lung cancer is not as well understood as that for CDDP. Some authors have suggested that the effect of PAX in *in vitro* studies (12, 25, 35), *in vivo* animal studies (37), and human studies (21, 38) of lung cancer is independent of and possibly even suppressed by p53 protein viability. This *p53*-independence was especially apparent when low weekly doses of PAX were administered (21). This lack of effect at low doses is indirectly consistent with the current results, since in this study the greatest effect was seen at the highest concentration in our range of study (27nM, Figure 4B). Ling *et al*. stated that since wt-*p53* gene results in cell cycle arrest at the G_1 phase, and PAX requires continuation of the cell cycle to act at the G_2/M phase,

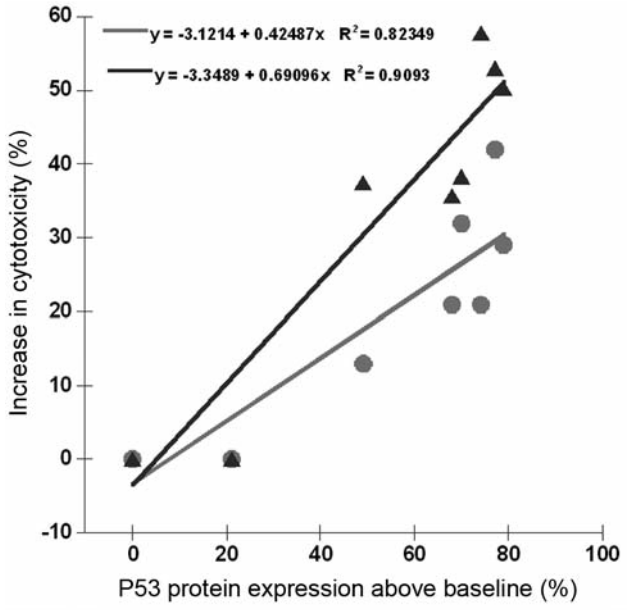

Figure 5. *Correlation between increase in p53 protein expression and increase in cytotoxicity as compared with untransfected control NSCLC cells. The graph expresses data from all three cell lines. There was a positive correlation of increase in CDDP (●) and PAX (▲) cytotoxicity to increased p53 protein expression; Pearson correlation for CDDP and PAX was R^2=0.823 and R^2=0.909, respectively (p<0.001).*

its effects are thwarted by the effects of the wt-*p53* gene (25). However, the current results suggest an enhanced response of NSCLC cells to PAX when transfected with wt-*p53* gene expressing a functional protein. PAX is theorized by some to have two mechanisms of action on cancer cell cytotoxicity: (i) *p53*-dependent G_2/M cell cycle arrest resulting in 'slow' apoptosis, and (ii) *p53*-independent prophase arrest resulting in 'rapid' apoptosis (39, 40). Some cancer cells, such as A549, are apoptosis reluctant, leading to mitotic arrest rather than apoptosis when treated with anticancer agents (41, 42). Mitotic arrest results in a depletion of the MDM2 (the *p53*-degrading) protein, thereby resulting in accumulation of p53 protein and G_1 cellular arrest ('slow apoptosis'), a potential mechanism for *p53*-dependent apoptosis with PAX treatment (41-44). The enhanced NSCLC cytotoxic response to PAX, after wt-*p53* or *CD-1* transfection, may be explained through the principle of 'slow' apoptosis in previously apoptosis-reluctant cells. NSCLC cells with mutated or deleted *p53* gene (H358 and H1299) do not posses this natural advantage of accumulated functional p53 protein resulting in 'slow' apoptosis when treated with PAX. Apoptosis-reluctance to mitotic inhibitors such as PAX may not be a predictable phenomenon, thus supporting the need for increased p53 protein in conjunction with drug treatment.

Elevated levels of functional p53 protein, conferred by *CD-1* gene transfection, enhanced the effect of both CDDP and PAX in NSCLC cells that naturally synthesize normal p53 protein (A549), and in those known to have mutated loss of p53 protein (H358, H1299). These results are supported by the known dependence of CDDP upon normal *p53* expression. However, the protein levels have not previously been directly correlated with enhanced efficacy of CDDP. A similar trend was seen between high p53 protein level and PAX enhancement (Figures 4B and 5). Extrapolated from the discussion above on the role of accumulated cellular p53 protein in apoptosis-reluctant cells, one might expect that A549 requires elevated protein levels to overcome its resistance to PAX. From the current study, it is not possible to suggest a similar correlation between elevated p53 protein levels and PAX treatment seen in the presumably apoptosis-sensitive cell lines (H358 and H1299). However, others have proposed that different cancer cells may exhibit different apoptosis thresholds (45). Wang *et al*. previously suggested that the amount of p53 protein may be critical for determining whether cells are directed to undergo DNA repair or be marked for apoptosis (46), with low p53 levels associated with increased failure of response to CDDP (47, 48). Achieving higher levels of functional p53 protein, as in the current investigation, may shift the balance between apoptosis inducers and apoptosis inhibitors in cancer cells. By promoting apoptosis in cancer cells, their responsiveness to chemotherapeutic agents may be improved. Supporting evidence is provided by *FUS1*, a tumor suppressor gene located on chromosome 3 (3p21.3) that produces down-regulation of MDM2, accumulation of p53 and activates the Apaf-1-dependent apoptosis pathway (30). Exogenous expression of FUS1 by nanoparticle-mediated gene transfer enhanced the sensitivity of lung cancer cells to CDDP (30).

There are certain limitations in this preliminary study in correlating p53 protein concentrations with chemo-sensitization. The current results were not corrected for the phase of the cell cycle in which the population of cancer cells existed when being treated with the drug. This may be important to differentiate the exact mechanism that is resulting in enhanced sensitivity, especially in the controversial role of PAX. If the proportion of cells in G_2/M phase is overly represented, then the percent of cells that seemed to be affected by the gene–PAX interaction could be artificially elevated. However, if the proportion of cancer cells in G_1 is greater, then in reality, the combination effect is underrepresented. Similar to the MTT assay, the Alamar Blue assay used in this study indirectly tests for cell viability rather than specifically quantifying levels of apoptotic proteins. However, both the metabolic assays correlate well to apoptosis and are accepted as an indirect mechanism of cell death. As alluded by Blagosklonny, detection of absolute death may not be necessary as prolonged cell cycle arrest itself, in the setting of elevated p53 proteins, may in fact result in eventual 'slow' apoptosis (41). Lack of significant PAX effect in the *CD-1*-transfected A549 and H358 cells may be a result of limited transfection time with the *p53* genes or short interval from

treatment to viability assay. Prolonged exposure to gene and/or drug may have allowed for enhanced death of the apoptotic-resistant cells, especially the A549 cells known to be resistant to initial PAX treatment, requiring a longer course for final cell death (41-42). This prolonged drug exposure of tumor cells may be better achieved in *in vivo* tumors that retain drug within the three-dimensional tumor.

We observed an enhanced response of NSCLC cells to two mechanistically differing chemotherapeutic agents when p53 protein expression was elevated beyond baseline expression. This enhanced response was achieved by transfecting NSCLC cells with the truncated wt-*p53* gene (*CD-1*), which also resulted in higher p53 protein expression in comparison with whole wt-*p53* gene transfection. NSCLC cells expressing p53 proteins at supra-physiologic levels resulted in enhanced chemosensitivity to both CDDP and PAX, although these cells were previously suggested to respond to chemotherapy in a manner independent of *p53* status. These findings suggest a role for *CD-1* transfection used in conjunction with chemotherapy for treatment of human NSCLC tumors that are early in tumor progression or chemoresistant due to *p53* mutations.

Conclusion

Transfecting NSCLC cells with the *CD-1* gene construct results in a higher p53 protein expression and better cytotoxic response to anticancer drugs CDDP and PAX. Response to anticancer drugs is improved with *CD-1* gene transfection compared with wt-*p53* gene transfection *in vitro*. We also show that PAX function in NSCLC cell death is improved with functional p53 protein as a consequence of either wt-*p53* or *CD-1* gene transfection. Further studies to demonstrate this principle *in vivo* are necessary.

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