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Preclinical report

Selective chemosensitization of rhabdomyosarcoma cell lines following wild-type p53 adenoviral transduction

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Rhabdomyosarcoma (RMS) cell lines were transduced with an adenoviral vector containing the wild-type p53 (wtp53) cDNA (Ad-p53) and then exposed to four cytotoxic agents: actinomycin D, vincristine, 5-fluorouracil and bleomycin. Potentiation of cytotoxicity following wild-type p53 expression varied from 0- to 20-fold for different drugs and between cell lines. It appeared that alveolar RMS cells ($n=2$) were more susceptible to p53-mediated chemosensitization than embryonal RMS cells ($n=3$), although this was independent of pax3–FKHR expression. Overall, cells that were most chemosensitive prior to Ad-p53 exposure were those that were most susceptible to p53 potentiation of cytotoxicity. The different results obtained with these RMS cell lines does not appear to be related to expression of pax3–FKHR, p21, Bax or Bcl-2 but may in part be due to differential regulation of p53 target genes, such as MDM2. In conclusion, exogenous wild-type expression selectively chemosensitizes RMS cells to cytotoxic agents. However, expression of transcriptionally active wtp53 does not predict a chemosensitive phenotype. [© 2002 Lippincott Williams & Wilkins.]

Key words: Drug sensitivity, p53, pax3–FKHR, rhabdomyosarcoma.

Introduction

Wild-type p53 (wtp53) can induce apoptosis following exposure to DNA-damaging agents and during deregulated cell growth, a phenotype that mutant p53 proteins no longer posses.[1,2] Consequently, the high proportion of human tumors that contain p53 mutations are often more aggressive with a poor prognosis compared to those with a wtp53 gene sequence.[3,4] The ability of wtp53 to induce apoptosis has been investigated in various model systems. Exogenous p53 expressed following viral vector transduction has been demonstrated to reduce cell proliferation in a variety of tumor cell lines *in vitro* ranging from lung and breast carcinoma to lymphomas and leukemias.[5] In addition, p53 expression studies in human tumor xenografts have resulted in suppression of tumor growth.[5] These preclinical data have been used to support the development of wtp53 cancer gene therapy clinical protocols. Phase I clinical trials utilizing p53 viral vectors are underway and to date have yielded promising results demonstrating minimal toxicity to normal tissues.[6,7]

Exogenous expression of wtp53 can mediate apoptosis of tumor cells without exposure to toxic stimuli, but it can also enhance apoptosis induced following exposure to ionizing radiation and chemotherapeutic drugs.[8–12] Less work has been carried out investigating this kind of combination therapy; however, it is likely that future cancer gene therapy protocols will be combined with more conventional chemotherapeutic regimens.

Although there are numerous published reports demonstrating the association of wtp53 and apoptosis, there are also several contradictory reports.[10,13–15] There appears to be a variation in responses observed dependent upon the tumor types evaluated and the cytotoxic agents to which they were exposed.

In this study, tumor cell lines of the same histiotype were evaluated to investigate the variation in wtp53 responses within one tumor type. Pediatric rhabdomyosarcoma (RMS) cell lines were chosen because this tumor type has a relatively low frequency of p53 mutations ranging from 5 to 30%[16–19] and is generally classed as chemosensitive.[20] It is possible that wtp53 expression

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contributes to its chemosensitive phenotype and wtp53 gene therapy may be useful to sensitize the subset of tumors that express mutant p53. Pediatric cancers have generally been overlooked for these types of analyses. In fact there is only one published report investigating the effect of wtp53 expression on chemosensitivity in a single RMS cell line, Rh30.[21] Gibson *et al.* reported wtp53-mediated sensitization to actinomycin D (ActD), 5-fluorouracil (5-FU) and bleomycin (Bleo), but not to etoposide, cisplatin and vincristine (VCR).[21]

This study demonstrates that not all RMS cells can be chemosensitized by wtp53 expression and that there are drug-specific effects in the cells that do display p53-enhanced cytotoxicity. Data presented here suggest that a chemosensitive phenotype may predict an enhanced wtp53 response and that an attenuated p53 response could in part be associated with elevated MDM2 expression.

Materials and methods

Cell lines and their p53 status

The RMS cell lines used in this study and their p53 status have been previously described by Taylor *et al.*[19] CT-TC cells were obtained from Dr H Hosoi (Kyoto Prefectural University of Medicine, Kyoto, Japan). They contain a deletion of nucleotides 1236–1239 (based upon the numbering by Buchman *et al.*[22]) resulting in a premature stop codon. RD cells were obtained from the ATCC (Rockville, MD) and contain a codon 248 Arg–Trp mutation. JR1 cells were established at the Institute for Child Health, London, UK.[23] They were obtained from Dr Peter Dias (Imgenex, San Diego, CA) and also contain a codon 248 Arg–Trp mutation. Rh41 and Rh30 cells were established at St Jude Children's Research Hospital (SJCRH) by Dr Peter Houghton. Rh41 cells contain a deletion of nucleotides 1001–1013 of the p53 cDNA and express no full-length p53 protein due to a premature stop codon. Rh30 cells contain a 273 Arg–Cys p53 mutation.

Adenoviral vectors and cell transduction

Ad-p53 (Av1p53) was provided by Genetic Therapy (a Novartis Company, Gaithersberg MD).[24] Ad-VC was provided by Dr Janet Houghton (SJCRH). Cells were transduced with the adenoviral vectors for 24 h at an appropriate m.o.i., prior to addition of fresh media. The m.o.i. was calculated based upon the number of adenoviral plaque forming units as determined by titering on 293 cells. Cells (2×10^6/well) were plated into six-well plates and after an overnight attachment period serial dilutions of the adenoviral vectors were added to the cells for 1 h in 1 ml of media. Cells were then overlayed with 1% SeaPlaque agarose (Biowhittaker, Rockland, ME) and plaques counted following a 2-week incubation period at 37°C, 10% CO_2/90% air.

Antibodies and Western analysis

Cell extracts were prepared and Western analyses were carried out as previously described.[25] The p53 monoclonal DO1-HRP, the Bax polyclonal and the Bcl-2 monoclonal antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The p21 monoclonal was obtained from PharMingen (San Diego, CA) and the MDM2 monoclonal (IF2) was obtained from Oncogene Science (Cambridge, MA).

Cytotoxic agents

ActD was obtained from Merck (West Point, PA), VCR was obtained from Eli Lilly (Indianapolis, IN), 5-FU was obtained from Pharmacia (Kalamazoo, MI) and Bleo was obtained from Mead Johnson Oncology Products (a Brystol-Myers Squibb Company, Princeton, NJ).

Growth curve and growth inhibition assays

To measure the rate of cell growth, 1×10^5 cells/well were plated into six-well plates and the total numbers of cells in each well were counted every 1–3 days. Growth inhibition assays were carried out by plating cells into six-well plates as described above. Following a 24-h attachment period, cells were exposed to a range of concentrations of adenovirus or cytotoxic agents. The total numbers of cells in each well were counted after the untreated cells had doubled 3 times. Data were calculated as a percentage of the cells in the untreated wells.

MTT assay

Cells were plated into 96-well plates at a density that allowed exponential cell growth but that did not result in a saturated A_{490} value following three cell

divisions. The number of cells plated for each of the cell lines was for CT-TC 1.5×10^4, for RD 2×10^3, for JR1 4×10^3 and for Rh41 7×10^3. After overnight attachment the cells were exposed to Ad-p53 or Ad-VC. The following day the virus was removed and the cells were treated with varying concentrations of cytotoxic agents. After a period of three cell doublings fresh medium was added to the cells prior to addition of Cell Titer 96 AQ_{ueous} One Solution (Promega, Madison WI). Following a 2-h incubation at 37°C, the plates were measured at A_{490} using an MRX microtiter plate reader (Dynex, Chantilly VA).

Production and characterization of pax3–FKHR-expressing clones

pXFKHD is a pax3–FKHR cDNA expression vector obtained from Jack Sublett and Dr David Shapiro (SJCRH). This plasmid was transfected into JR1 cells using the ProFection Mammalian Transfection System from Promega. Following selection and expansion of clones resistant to 200 μg/ml G418, RT-PCR confirmed expression of the pax3–FKHR fusion transcript. Total RNA was prepared using the RNAqueous kit from Ambion (Austin, TX) and RT-PCR was carried out using the Access RT-PCR kit from Promega. The primers used were those previously described by Shapiro *et al.*[26] The size of the PCR product generated was 358 bp. Transcriptional activity of pax3–FKHR in the transfected cells was confirmed by transient transfection with pGL2-R59, a pax3-responsive luciferase reporter plasmid obtained from Mr Sublett and Dr Shapiro. Forty-eight hours after transfection cells were harvested and luciferase assays carried out using Promega's Luciferase Assay Reagent. Data were presented as relative light units (RLU)/mg protein.

Results

Ad-p53 transduction of RMS cell lines

The adenoviral vector Ad-p53 and a control virus Ad-VC were used to transduce four RMS cell lines in growth inhibition assays. Adenoviral dose–response curves were obtained and the results for Ad-p53 are shown in Figure 1. The p53 adenovirus resulted in a reduced cell number of all the lines but to varying degrees. The m.o.i.s resulting in a 50% growth inhibition (IC_{50}) for each of the four lines were 0.1 for Rh41, 1 for RD, 6 for CT-TC and 8 for JR1. The equivalent IC_{50} values for Ad-VC were 6 for Rh41, and >30 for RD, CT-TC and JR1 (data not shown). In order to carry out experiments to investigate the influence of wtp53 expression on drug sensitivity we chose m.o.i.s that would allow equivalent expression of p53 protein and/or transcriptional activity as measure by p21 induction. Figure 2 contains Western analyses for p53 and p21 expression in the four cell lines following transduction with Ad-VC and Ad-p53 adenoviral vectors. Because JR1 and RD express high levels of mutant p53 protein, transcriptional activation of p21 was compared as a measure of wtp53 function in these lines. The m.o.i.s of 3 for JR1 and 1

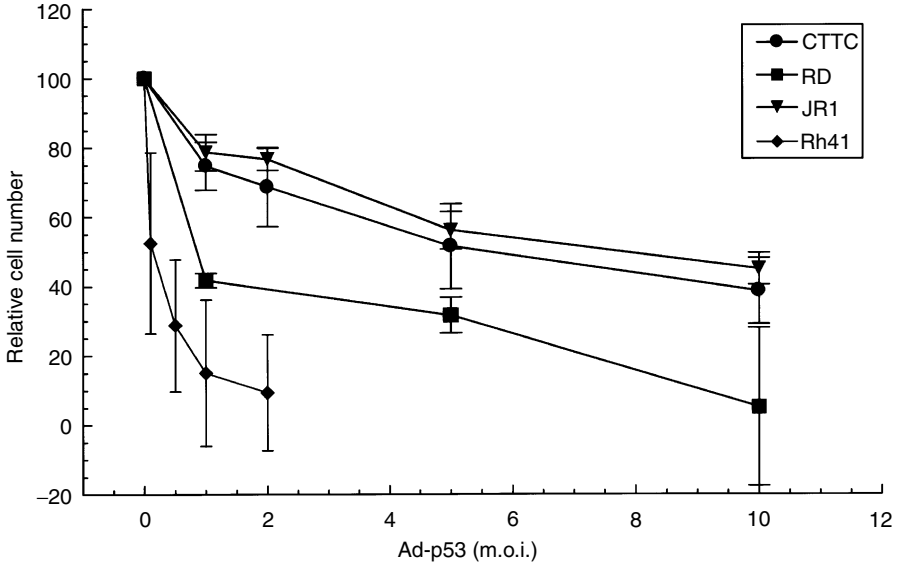

Figure 1. Growth inhibition analysis of RMS cell lines exposed to increasing amounts of Ad-p53 adenoviral vector.

for RD resulted in identical levels of p21 expression and reduced cell growth rates of 33 and 40%, respectively (Figure 2 and Table 1). The growth of CT-TC and Rh41 was slowed by 33 and 17% following transduction with m.o.i.s of 1 and 0.1, respectively (Table 1). Even though wtp53 expression resulted in a slowing of cell growth, all lines went through three cell divisions within 9 days. Ad-VC transduction affected the growth rate of only the RD cells (Table 1).

Exposure of RMS cell lines to Ad-p53 and cytotoxic agents

Dose–response curves following addition of four cytotoxic agents were generated for the four cell lines with and without Ad-VC and Ad-p53 transduction. The cytotoxic agents chosen were ActD, VCR, 5-FU and Bleo.[27,28] ActD, 5-FU and Bleo were the drugs to which the greatest degree of sensitization had been previously observed following induction of wtp53 in Rh30 cells.[21] VCR is a mitotic spindle poison and was chosen as an example of a non-DNA-damaging agent. ActD inhibits both topoisomerase I and II, thereby generating both single- and double-strand DNA breaks, and at higher concentrations it can also inhibit RNA synthesis. 5-FU is an antimetabolite that inhibits thymidine synthesis. It causes single- and double-strand DNA breaks by depleting dTTP pools and by misincorporating FU or U bases into DNA. Bleo is a radiomimetic that generates free radicals causing double strand breaks in DNA.

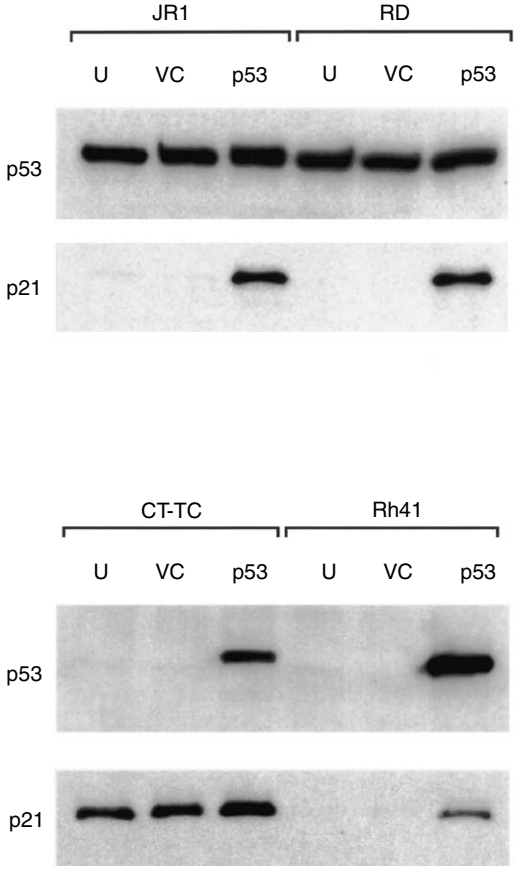

Figure 2. Western analysis of the RMS cell lines 24 h following transduction with either VC (Ad-VC, vector control adenovirus) or p53 (Ad-p53 adenovirus). U represents non-transduced cells. m.o.i.s of adenovirus were 3 for JR1,1 for CTTC and RD, and 0.1 for Rh41. These m.o.i.s were chosen as described in the text.

Table 1. Time (days) for cells to double 3 times with and without exposure to adenoviral vectors

Cell line	Untreated	Ad-VC	Ad-p53	%[a]
CT-TC	6	6	9	33
RD	3	4	5	40
JR1	2	2	3	33
Rh41	5	5	6	17

[a]Percentage reduction in growth rate following Ad-p53 transduction.

Cells were exposed to the adenoviral vectors for 24 h prior to addition of the cytotoxic agents. Cytotoxicity of the cells was then evaluated after the cells had passed through three cell doublings. Exposure for a fixed number of cell divisions allows a comparison of the data generated from cell lines growing at different rates. Each experiment was performed at least in triplicate and the concentration of drug that resulted in a 50% reduction in cell number (IC_{50}) was calculated.

Table 2 contains the relative IC_{50} values for Ad-VC transduced cells relative to those of the same cell line exposed to Ad-p53. A value of 1 indicates no

Table 2. Relative IC_{50} values of cells following transduction with Ad-p53 compared to cells transduced with Ad-VC and exposed to cytotoxic agents

Cell line	ActD	VCR	5-FU	Bleo
ERMS				
CT-TC	0.52	1.03	0.48	0.32
RD	0.53	0.79	0.41	**0.05**
JR1	0.47	0.73	0.61	0.86
ARMS				
Rh41	**0.26**	0.76	**0.15**	**0.18**
Rh30	**0.20**	1.00	**0.23**	**0.12**

Values in bold highlight >3-fold chemosensitization following wild-type p53 expression. Rh30 data are taken from Gibson *et al.*[21] and are included for comparison. ERMS (embryonal RMS) cell lines and ARMS (alveolar RMS) cell lines are as indicated.

chemosensitization of the cells by wtp53. The values in bold are those that demonstrated greater than a 3-fold p53-mediated chemosensitization. Representative graphs of JR1 and RD cells exposed to Bleo with and without Ad-p53 transduction are shown in Figure 3. The degree of p53-mediated chemosensitization observed for the different lines varied from zero for CT-TC cells exposed to Ad-p53 and VCR to a 20-fold potentiation of cytotoxicity for RD cells exposed to Ad-p53 and Bleo. Exposure to Ad-p53 did not significantly influence the IC_{50} values for VCR in any of the cell lines. Rh41 demonstrated at least a 4- to 7-fold potentiation of cytotoxicity to ActD, 5-FU and Bleo following p53 expression. These data are similar to those previously published for another alveolar RMS cell line, Rh30.[21] JR1, RD and CT-TC are all cell lines derived from embryonal RMS tumors. These cell lines were chemosensitized only 2- to 3-fold to most of the drugs following wtp53 expression. The exceptions were JR1, which demonstrated less than 2-fold difference in IC_{50} values following exposure to 5-FU and Bleo, and RD which was 20-fold more sensitive to Bleo following expression of wtp53 (Table 2 and Figure 3).

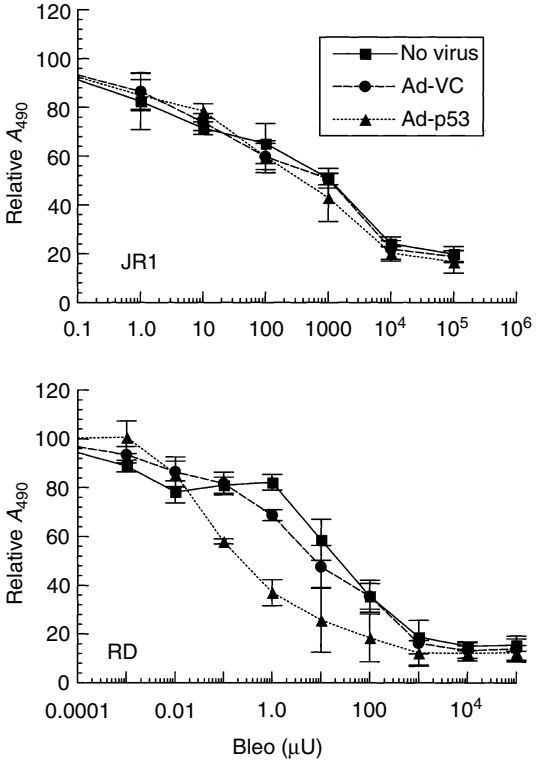

Figure 3. Dose–response curves for JR1 and RD cells following exposure to Ad-VC or Ad-p53 and Bleo. Relative A_{490} values were generated by the MTT assays. wtp53 expression chemosensitized RD cells to the cytotoxic effects of Bleo, but JR1 cell Bleo sensitivity was unaffected by p53 expression.

Table 3. IC_{50} values of cells prior to adenoviral exposure

Cell line	ActD (nM)	VCR (nM)	5-FU (μM)	Bleo (μU)
ERMS				
CT-TC	1.65	0.23	5.42	283
RD	0.99	0.57	5.02	**44**
JR1	3.50	0.76	23.6	1011
ARMS				
Rh41	**1.65**	0.26	**2.58**	**44**
Rh30	**0.10**	0.30	**4.30**	**345**

The experiments that generated >3-fold p53-mediated chemosensitization as shown in Table 2 are again highlighted in bold. Bleo concentration is measured in micro units (μU).

Table 3 contains IC_{50} values for the cell lines prior to adenovirus transduction. These data demonstrate that in general the chemosensitive cell lines were those that were most likely to become sensitized by wtp53 expression. For example, the Bleo IC_{50} value for RD cells was 44 μU and these cells could be sensitized 20-fold to Bleo following wtp53 expression. In comparison, JR1 demonstrated no significant change in chemosensitivity following wp53 expression and the IC_{50} value for Bleo in these cells was 1011 μU (Table 3 and Figure 3).

Expression of pax3–FKHR in JR1 cells

Alveolar RMS tumors are characterized by a t(2:13) translocation resulting in expression of a PAX3–FKHR fusion protein. We evaluated whether expression of this fusion protein could influence chemosensitization induced by wtp53 expression. The PAX3–FKHR cDNA contained in plasmid pXFKHD was expressed in the embryonal cell line JR1 that does not contain the t(2;13) translocation. Cells were selected with 200 μg/ml G418 and individual clones isolated and expanded. Two clones, PxF7 and PxF10 were demonstrated to express the fusion transcript by RT-PCR (Figure 4A). Two G418 resistant clones of JR1 that had not been transfected with pXFKHD were analyzed as negative controls. To confirm expression of pax3–FKHR, PxF7 and PxF10 were transiently transfected with pGL-R59, a luciferase reporter gene plasmid containing a pax3 responsive promoter. Forty-eight hours after transfection cells were harvested for luciferase assays and the RLU/mg protein calculated. Figure 4(B) demonstrates that both PxF7 and PxF10 can activate the pax3-responsive promoter confirming functional pax3–FKHR expression.

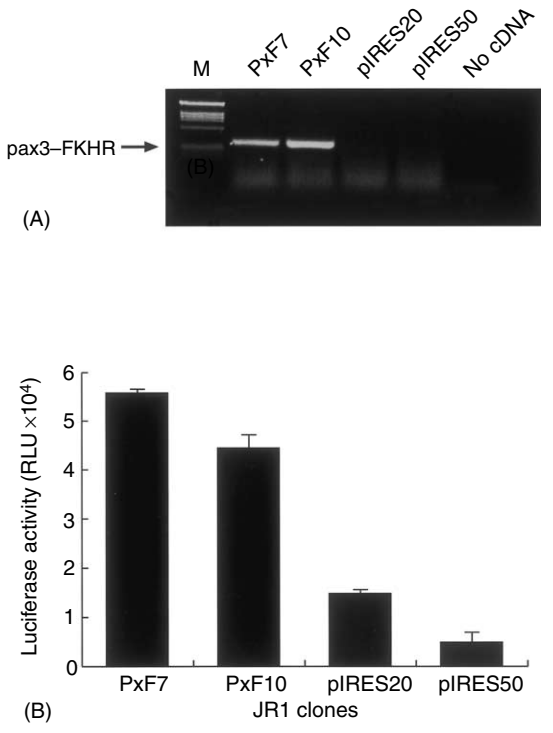

Figure 4. Characterization of pax–FKHR transfected JR1 cells. (A) RT-PCR analysis demonstrating expression of the fusion transcript in clones PxF7 and PxF10. PIRES20 and pIRES50 are G418-resistant control JR1 cells. M represent λ*Hin*dIII DNA molecular weight markers. (B) Results of luciferase assays of pax3–FKHR-expressing JR1 clones transiently transfected with pGL2-R59, a pax3-responsive luciferase reporter plasmid. Elevated luciferase activity measured in PxF7 and PxF10 cells demonstrated pax-responsive promoter activity in these JR1 clones.

The pax3–FKHR-expressing cells were exposed to Ad-p53 and ActD to determine whether expression of the fusion protein could enhance p53-mediated chemosensitization of JR1 cells. Ad-p53 exposure at an m.o.i. of 3 was for 24 h prior to addition of ActD to allow expression of wtp53. Figure 5 demonstrates that although PxF10 cells were chemosensitized 2-fold to ActD upon expression of wtp53, this was no greater potentiation of cytotoxicity compared to the control cells, pIRES20 (Figure 5) or parental JR1 cells (Table 2). These data suggest that the pax3–FKHR fusion protein plays no role in p53-mediated chemosensitization of RMS cells.

Comparison of JR1 and RD cell lines

The wtp53 expression in the embryonal RMS cell lines did not potentiate cytotoxicity of any of the drugs greater than 3-fold with the exception of RD cells following exposure to Bleo. Ad-p53 and Bleo exposure chemosensitized RD cells by 20-fold. JR1 cells demonstrated the smallest change in chemosensitivity of less than 2-fold to this virus/drug combination. These two cell lines express the same p53 codon 248 mutation resulting in an amino acid change from arginine to tryptophan. In order to investigate the potential mechanism for the difference in wtp53-mediated chemosensitization between these two cell lines, p53-regulated expression of two apoptosis regulating proteins Bax and Bcl-2 was analyzed. Following exposure to Ad-p53 for 24 h and for 1, 2 and 3 cell doublings, cells were harvested for Western analysis. Cells were also harvested at 1, 2 and 3 cell doublings following exposure to an IC_{50} concentration of Bleo.

Figure 6 demonstrates that Bax expression was induced equally by wtp53 in both cell lines, but that there was no effect on Bcl-2 expression. The same Western membranes were also probed with an anti-MDM2 antibody (Figure 7A). These results demonstrate that MDM2 expression was differentially induced by wtp53 in RD and JR1 cells. RD cells were sensitized 20-fold to Bleo and induced MDM2 to a much lower level and for a shorter time period compared to JR1 cells. Basx and p21 were induced equally by wtp53 in RD and JR1 cells (Figures 2 and 6), but MDM2 expression was induced to a higher level in JR1 cells compared to RD (Figure 7A). The different levels of MDM2 expression could be regulated by $p14^{ARF}$ since $p14^{ARF}$ is expressed at a higher level in RD cells compared to JR1 (Figure 7B).

Discussion

In order to compare the effect of wtp53 expression on chemosensitivity, Ad-p53 was used to transduce RMS cells lines. Equivalent levels of p53 expression were generated by transducing cells with different m.o.i.s (Figure 2). The m.o.i. of Ad-p53 chosen allowed cell division which was important in order to observe the action of the different cytotoxic agents. Even though the cell lines expressed approximately equivalent levels of wtp53, the potentiation of cytotoxicity to the different drugs varied (Table 2 and Figure 3). Expression of wtp53 did not significantly influence the cytotoxicity of VCR, the mitotic spindle inhibitor, in any of the cell lines. However, the cytotoxicity of the other three DNA damaging agents was potentiated by at least 3-fold in at least one of the cell lines. The alveolar RMS cell line Rh41 was the

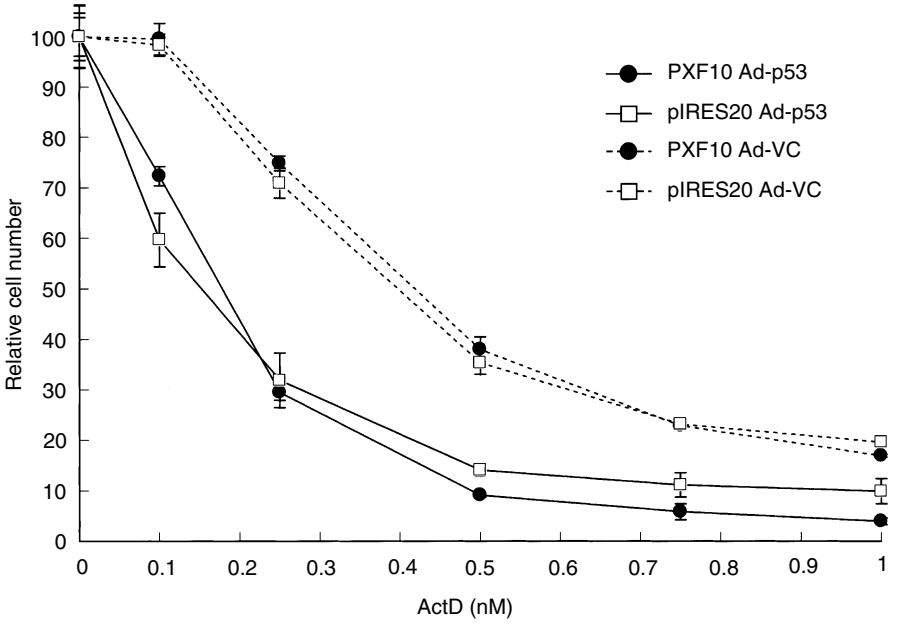

Figure 5. Cytotoxicity assays of pax3–FKHR-expressing PxF10 cells and vector control pIRES20 cells exposed to Ad-VC or Ad-p53 for 24 h prior to exposure to ActD. Data demonstrate that although the cells expressing p53 were chemosensitized to ActD, there was no significant difference compared to the pIRES20 cells that did not express the pax–FKHR fusion protein.

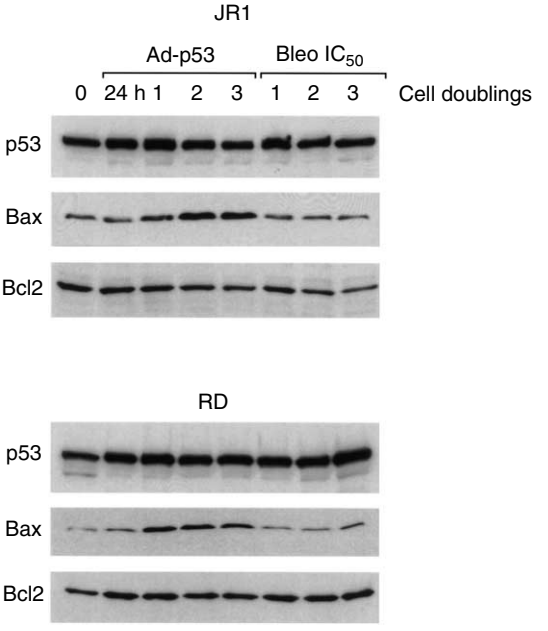

Figure 6. Western analysis of JR1 and RD cells following exposure to either Ad-p53 or Bleo. Cells were harvested after 24-h exposure to Ad-p53, and also following 1, 2 and 3 cell divisions. Following exposure to Bleo cells were harvested following 1, 2 and 3 cell divisions. The membrane was sequentially probed with p53, Bax and Bcl-2 antibodies.

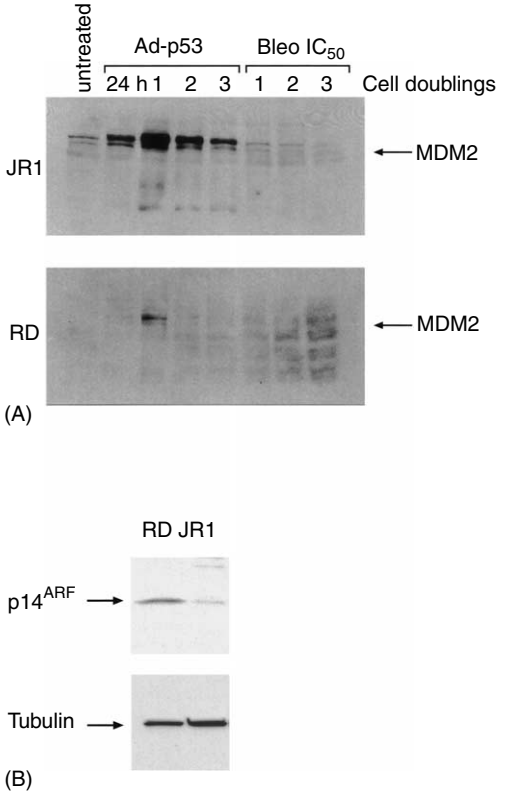

Figure 7. (A) The same Western as described in Figure 6 was probed with anti-MDM2 antibodies. (B) Cell extracts of untreated exponentially growing RD and JR1 cells were probed with anti-$p14^{ARF}$ and tubulin antibodies.

most affected by wtp53 expression as demonstrated by chemosensitization to ActD, 5-FU and Bleo following Ad-p53 transduction. Alveolar RMS tumors are characterized by containing a t(2;13) translocation that results in expression of a pax3–FKHR fusion protein. We hypothesized that expression of this fusion protein might influence the ability of wtp53 to chemosensitize cells to cytotoxic agents. To test this, JR1 embryonal RMS cells were transfected with the pax3–FKHR cDNA, and clones expressing this protein were developed and characterized (Figure 4). These pax3–FKHR-expressing clones were no more sensitive to ActD than the parental JR1 cells or to G418-resistant vector control cells, suggesting that the pax3–FKHR fusion protein does not play a role in p53-mediated chemosensitization of RMS cells (Figure 5). However, it is possible that alveolar RMS cells express other factors that influence the action of wtp53.

The cell lines that were most chemosensitive to individual cytotoxic agents prior to Ad-p53 exposure were generally those that displayed the greatest chemosensitization following wtp53 expression (Tables 2 and 3). These data suggest that cells in which wtp53 expression could not potentiate cytotoxicity are generally more resistant to induction of apoptosis. A comparison of RD and JR1 cells revealed that even though p21 and Bax were induced to equivalent levels by exogenous wtp53 expression, MDM2 was induced to a higher level and for a longer time period in the drug-resistant JR1 cells (Figure 7). Therefore, even though wtp53 was transcriptionally active, enhanced MDM2 expression may function to inhibit p53-mediated chemosensitization of JR1 cells. These data suggest that wtp53 transcriptional activity is dependent on the cell type and that the level of induction of different p53 target genes may vary. Alternatively, other cellular factors may influence MDM2 protein stability.

$p14^{ARF}$ is known to bind MDM2[29] and is expressed at a higher level in RD cells compared to JR1 (Figure 7B). It is possible that $p14^{ARF}$ may play a role in influencing MDM2 protein stability in RD cells resulting in low MDM2 expression. It is likely that elevated expression of MDM2 in JR1 cells resulted in inhibition of wtp53 chemosensitization compared to RD cells. MDM2 has been demonstrated to influence p53-mediated apoptosis in other model systems, e.g. Sato *et al.*[30] demonstrated enhanced p53-mediated drug-induced apoptosis using antisense oligonucleotides targeted to MDM2 and Conforti *et al.*[31] demonstrated that lack of MDM2 induction resulted in enhanced p53-mediated apoptosis. Cocker *et al.* have also shown that MDM2 may enhance expression of P-glycoprotein and confer multidrug resistance in RMS cells.[32] However, the function of MDM2 must be cell specific because we have been unable to directly demonstrate that MDM2 expression inhibits wtp53-mediated chemosensitivity in other RMS cells (data not shown). In addition, because RD cells were not sensitized 20-fold to drugs other than Bleo, additional cellular factors appear to be important in influencing the chemosensitizing phenotype of wtp53.

Bcl-2 expression has previously been shown to be down-regulated by wtp53.[33] However, Ad-p53 did not influence the expression of Bcl-2 in either JR1 or RD cells, indicating that this protein was not responsible for the differences in p53-mediated chemosensitivity observed for the two cell lines (Figure 6). The observation that Bax was induced equally by p53 in both RD and JR1 cells suggests that proteins downstream from Bax in the apoptotic pathway may play a role in inhibiting p53-mediated Bleo sensitization in JR1 cells.

Conclusion

In conclusion, exogenous wtp53 expression in RMS cells selectively potentiates the action of cytotoxic agents and expression of transcriptionally active wtp53 does not predict a chemosensitive phenotype. However, a chemosensitive cellular phenotype may predict an enhanced wtp53-mediated cytotoxic response. The varied results obtained with the cytotoxic agents in these RMS cell lines does not appear to be related to expression of the pax3–FKHR fusion protein, p21, Bax or Bcl-2, but may in part be due to differential regulation of p53 target genes, such as MDM2. Further work will be required to understand the mechanism of how wtp53-mediated apoptosis is inhibited. Identification of novel inhibitory factors may facilitate future predictions of cytotoxic responses in the presence or absence of wtp53 expression.

Acknowledgments

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References

1. Lane DP. p53, Guardian of the genome. *Nature* 1992; **358**: 15–6.

2. Zambetti GP, Levine AJ. A comparison of the biological activities of wild-type and mutant p53. *FASEB J* 1993; **7**: 855–65.
3. Levine AJ, Momand J, Finlay CA. The p53 tumor suppressor gene. *Nature* 1991; **351**: 453–5.
4. Levine AJ. p53, the cellular gatekeeper for growth and division. *Cell* 1997; **88**: 323–31.
5. Nielsen LL, Maneval DC. p53 tumor suppressor gene therapy for cancer. *Cancer Gene Ther* 1998; **5**: 52–63.
6. Schuler M, Rochlitz C, Horowitz JA, *et al.* A phase I study of adenovirus-mediated wild-type p53 gene transfer in patients with advanced non-small cell lung cancer. *Hum Gene Ther* 1998; **9**: 2075–82.
7. Swisher SG, Roth JA, Nemunaitis J, *et al.* Adenovirus-mediated p53 gene transfer in advanced non-small-cell lung cancer. *J Natl Cancer Inst* 1999; **91**: 763–71.
8. Lee JM, Bernstein A. p53 mutations increase resistance to ionizing radiation. *Proc Natl Acad Sci USA* 1993; **90**: 5742–6.
9. Lowe SW, Ruley HE, Jacks T, Houseman DE. p53-dependent apoptosis modulates the cytotoxicity of anticancer drugs. *Cell* 1993; **74**: 957–67.
10. Fan S, El-Diery WS, Bae I, *et al.* p53 gene mutations are associated with decreased sensitivity of human lymphoma cells to DNA damaging agents. *Cancer Res* 1994; **54**: 5824–30.
11. Kohn KW, Jackman J, O'Connor PM. Cell cycle control and cancer chemotherapy. *J Cell Biochem* 1994; **54**: 440–52.
12. Li JH, Lax SA, Kim J, Klamut H, Liu FF. The effects of combining ionizing radiation and adenoviral p53 therapy in nasopharyngeal carcinoma. *Int J Radiat Oncol Biol Phys* 1999; **43**: 607–16.
13. Labrecque S, Matlashewski GJ. Viability of wild-type p53-containing and p53 deficent tumor cells following anticancer treatment: the use of human papilloma virus E6 to target p53. *Oncogene* 1995; **11**: 387–92.
14. Slichenmeyer WJ, Nelson WG, Slebos RJ, Kastan MB. Loss of a p53-associated G_1 checkpoint does not decrease cell survival following DNA damage. *Cancer Res* 1993; **53**: 4164–8.
15. Vinyals A, Peinado MA, Gonzalez-Garrigues M, Monzo M, Bonfil RD, Fabra A. Failure of wild-type p53 gene therapy in human cancer cells expressing a mutant p53 protein. *Gene Ther* 1999; **6**: 22–33.
16. Felix CA, Kappel CC, Mitsudomi T, *et al.* Frequency and diversity of p53 mutations in childhood Rhabdomyosarcoma. *Cancer Res* 1992; **52**: 2243–7.
17. Mulligan LM, Matlashewski GJ, Scrable HJ, Cavenee WK. Mechanisms of p53 loss in human sarcomas. *Proc Natl Acad Sci USA* 1990; **87**: 5863–7.
18. Kusafuka T, Fukuzawa M, Oue T, Komoto Y, Yoneda A, Okada A. Mutation anlaysis of p53 gene in childhood malignant solid tumors. *J Pediat Surg* 1997; **32**: 1175–80.
19. Taylor AC, Shu LL, Danks MK, *et al.* p53 mutation and MDM2 amplification frequency in pediatric rhabdomyosarcoma tumors and cell lines. *Med Pediat Oncol* 2000; **35**: 96–103.
20. Pappo AS, Shapiro DN, Crist WM, Maurer HM. Biology and therapy of pediatric rhabdomyosarcoma. *J Clin Oncol* 1995; **13**: 2123–39.
21. Gibson AA, Harwood FG, Tillman DM, Houghton JA. Selective sensitization to DNA damaging agents in a human rhabdomyosarcoma cell line with inducible wild-type p53 overexpression. *Clin Cancer Res* 1998; **4**: 145–52.
22. Buchman VL, Chumakov PM, Ninkina NN, Samarina OP, Georgiev GP. A variation in the structure of the protein-coding region of the human p53 gene. *Gene* 1988; **70**: 245–52.
23. Clayton J, Pincott JR, van den Berghe JA, Kemshead JT. Comparative studies between a new human rhabdomyosarcoma cell line, JR-1 and its tumor of origin. *Br J Cancer* 1986; **58**: 83–90.
24. Pirollo KF, Hao Z, Rait A, *et al.* p53 mediated sensitization of squamous cell carcinoma of the head and neck to radiotherapy. *Oncogene* 1997; **14**: 1735–46.
25. McPake CR, Tillman DM, Poquette CA, George EO, Houghton JA, Harris LC. Bax is an important determinant of chemosensitivity in pediatric tumor cell lines independent of Bcl-2 expression and p53 status. *Oncol Res* 1998; **10**: 235–44.
26. Shapiro DN, Sublett JE, Li B, Downing JR, Naeve CW. Fusion of *PAX3* to a member of the forkhead family of transcription factors in human alveolar rhabdomyosarcoma. *Cancer Res* 1993; **53**: 5108–12.
27. Chabner BA, Collins JM. *Cancer chemotherapy: principles and practice*. Philadelphia, PA: Lippincott 1990.
28. Liu L. DNA topoisomerases: topoisomerase-targeting drugs. *Adv Pharmacol* 1994; **29B**: 1–315.
29. Sherr CJ. Tumor surveillance via the ARF-p53 pathway. *Genes Dev* 1998; **12**: 2984–91.
30. Sato N, Mizumoto K, Maehara N, *et al.* Enhancement of drug-induced apoptosis by antisense oligodeoxynucleotides targeted against Mdm2 and p21WAF1/CIP1. *Anticancer Res* 2000; **20**: 837–42.
31. Conforti G, Nardo T, D'Incalci M, Stefanini M. Proneness to UV-induced apoptosis in human fibroblasts defective in transcription coupled repair is associated with the lack of Mdm2 transactivation. *Oncogene* 2000; **19**: 2714–20.
32. Cocker H, Hobbs S, Tiffin N, Pritchard-Jones K, Pinkerton C, Kelland L. High levels of the MDM2 oncogene in pediatric rhabdomyosarcoma cell lines may confer multidrug resistance. *Br J Cancer* 2001; **85**: 1746–52.
33. Miyashita T, Harigai M, Hanada M, Reed JC. Identification of a p53-dependent negative response element in the *bcl-2* gene. *Cancer Res* 1994; **54**: 3131–5.

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