Adenoviral-Mediated Gene Therapy with Ad5CMVp53 and Ad5CMVp21 in Combination with Standard Therapies in Human Breast Cancer Cell Lines

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Abstract. Our objective was to determine the efficacy of adenoviral-mediated gene therapy with wild-type p53 or p21 in human breast cancer cells and investigate interactions with radiation and chemotherapy. Two human breast cancer cell lines, MDA-MB-231 and MDA-MB-435, both with p53 mutations, were transduced with adenoviral vectors containing wild-type p53 (Ad5CMV-p53) or p21/WAF1/CIP1 (Ad5CMV-p21), and the effects on growth were determined. Infection was combined with low-dose (1.4 - 3.7 Gy) irradiation to see if this would improve transduction efficiency and enhance numbers of cells killed. Transduction with either vector resulted in expression of p21/WAF1/CIP1 and growth inhibition, although Ad5CMV-p53 transduction produced greater growth inhibition than did Ad5CMV-p21. The cell lines differed in sensitivity to the vectors. The Ad5CMV-p53 vector in a multiplicity of infection (MOI) of 125 resulted in 50% to 80% inhibition of MDA-MB-231, while MOI 250 of the same vector resulted in 27% inhibition of MDA-MB-435. Infection with Ad5CMV-p21 produced modest growth inhibition in both cell lines (< 40% at MOI 200), although protein expression was detected at lower viral doses. Low dose γ-irradiation (1.4 to 3.7 Gy) was used to try and improve the rate of gene transfer. Modest increases in transduction efficiency and duration of expression of a vector containing β-galactosidase occurred in irradiated breast cancer cells. Radiation 24 hr before transduction with Ad5CMV-p53 increased the proportions of apoptotic MDA-MB-231 cells. The cells transduced with Ad5CMV-p21 were arrested in G1, yet when they were irradiated before adenoviral transduction, the overexpression of p21 protected the cells from the cytotoxic effects of the radiation. Clonogenic assays showed that Ad5CMV-p21 reduced the sensitivity of MDA-MB-231 to VP-16 and paclitaxel. Combining these drugs with Ad5CMV-p53 did not consistently or significantly decrease clonogenic survival. (received 20 April 2000; accepted 14 June 2000)

Keywords: gene therapy, chemotherapy, radiation, transfection, breast cancer, adenovirus

Introduction

Breast cancer is the most commonly diagnosed cancer in women in the U.S.A. Up to 50% of breast cancers have alterations in the tumor suppressor gene p53 [1-3]. The product of this gene is an important regulator of the cell cycle and of apoptosis of cells with DNA damage, for example, following exposure to irradiation or chemotherapeutic drugs [4-7]. Thus, the loss of wild-type p53 is thought to be a key step in cancer progression [8]. Other important elements in the cell cycle machinery that are commonly altered in breast cancer cells include the retinoblastoma susceptibility gene product (pRb) and cyclins and inhibitors involved in pRb phosphorylation. The p21/WAF1/CIP1 kinase inhibitor (hereafter termed p21) inactivates the cdk4-cyclin D complex, allowing pRb to retain control over S-phase transition [9,10]. One proposed mechanism of p53-mediated G1 arrest is through induction of p21 [11-13]. Other functions of p21 include interactions with proliferating cell nuclear antigen (PCNA) and cdk2 activity through cyclins E and A [9,10,14-17]. PCNA is essential for
nucleotide excision repair induced by UV radiation, and p21 is thought to be involved in this repair process through interactions with PCNA [18].

Adenoviral vectors are considered useful for gene therapy applications because of their relative ease of gene transfer and low toxicity [13,19]. In this study, adenoviral vectors expressing wild-type p53 and p21 were used to evaluate the effects of restoring expression of these genes on the growth of breast cancer cells with mutant p53. Further, we wished to determine whether combining the gene therapy with irradiation or chemotherapy resulted in greater therapeutic effect.

Some breast cancer cell lines, including the MDA-MB-435 line, were previously reported to have low transduction efficiency with adenoviral vectors [20]. Using ionizing radiation to enhance plasmid DNA or adenoviral vector transduction efficiency, as described by Zeng et al [21] and Stevens et al [22], we tested this approach with the breast cancer cell lines in an attempt to improve transduction with p53 and p21. As gene therapy is being introduced for the treatment of some types of cancer, the interactions of this modality with standard forms of therapy such as irradiation and chemotherapy should be evaluated to determine the consequences of combination therapy.

Materials and Methods

Cell culture. MDA-MB-231 and MDA-MB-435 cell lines were isolated from pleural effusions of patients with breast cancer at M. D. Anderson Cancer Center [23-25]. These breast cancer cell lines were grown in monolayer culture in Eagle's MEM supplemented with 5% FBS, 1 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, and MEM vitamin supplement (the supplemented medium is termed 5% CMEM).

Adenovirus infection. The recombinant adenovirus constructs contained the CMV promoter, wild-type p53 or p21 cDNA, and SV40 polyadenylation signal in a minigene cassette inserted into the E1-deleted region of modified Ad5, as described by Liu et al [19]. Viruses were kindly provided by Dr. Gary Clayman (Houston, TX). The control vector expressed β-galactosidase (Adβ-gal). MDA-MB-231 and MDA-MB-435 cells were plated at a concentration of 2 x 10^5 cells per well in 6-well plates. Serial dilutions of virus (Ad5CMV-p53, Ad5CMV-p21, or Adβ-gal) were made in 1 ml of 5% CMEM and added to 6-well plates 24 hr after plating the cells. The plates were shaken every 15 min for 1 hr, and then 3 ml of 5% CMEM was added to each well. The cells were then incubated at 37°C for the desired period of time. At various times, the cells were harvested from replicate or triplicate wells, and the numbers were counted using a hemocytometer. For clonogenic assays, cells were harvested 24 hr after viral infection and plated in 100-mm tissue culture plates, at 10^2, 10^3, or 10^4 cells per plate. After 10 days of incubation, the cells were fixed with methanol and stained with 0.1% methylene blue (dissolved in methanol), and the numbers of stained colonies per plate were counted.

β-gal staining. Transduction efficiency was assessed from the expression of the control β-gal gene. Breast cancer cells were plated in 6-well plates (2 x 10^5 per well), and 24 hr later the cells were transduced with multiplicities of infection (MOIs) of 0-1000 Adβ-gal. Twenty-four hr after transduction, the cells were washed with cold PBS and fixed in 0.5% glutaraldehyde. The cells were then incubated at room temperature for 5 min and washed twice with cold PBS. X-gal staining solution (1 M MgCl, 5 M NaCl, 1 M Tris pH 7.4, 30 mM potassium ferricyanide, 30 mM potassium ferrocyanide, and 2% X-gal [Ambion, Inc., Austin, TX] dissolved in DMF) was added in the amount of 2 ml per well. Twenty-four hr after staining, the numbers of cells in 5 high-power microscope fields per well were counted, and the percentages expressing β-gal were calculated.

Combination of irradiation and adenoviral transfection. The sensitivities of the breast cancer cell lines to irradiation were determined over a range of doses (1.44-14.67 Gy) from a 137cesium source (10,000 Ci giving 14.7 Gy/min, J.L. Shepherd and Assoc., Mark 1 Model 30). A gray (Gy) is absorption of 1 joule of energy/kg of tissue exposed. The doses chosen for further study, 1.44 and 3.7 Gy, resulted in 90% kill of MDA-MB-435 and MDA-MB-231, respectively, measured in 14-day clonogenic growth assays of the irradiated cells. The cells were irradiated 24 hr before, 1 hr before, or 24 hr after transduction.
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with adenovirus. At various times after treatment, the cells were harvested and counted and used for preparing protein lysates, for cell cycle analysis, or for clonogenic survival assays.

**Combination of chemotherapy and adenoviral transduction.** A clonogenic assay was used to assess the combination of drug and adenovirus. MDA-MB-231 cells were plated in 100-mm tissue plates and exposed to the virus (MOI 125) 24 hr after plating. Twenty-four hours after infection, VP-16 (5 \( \mu \)M), paclitaxel (1 \( \mu \)M), doxorubicin (0.1 \( \mu \)g/ml), or no chemotherapy agent were added. Cells were harvested 24 hr later for clonogenic assays as previously described.

**Flow cytometry for cell cycle analysis and annexin V expression.** Both floating and attached cells were collected from cultures of cells exposed to irradiation and adenovirus. For propidium iodide (PI) staining, 1 x 10^6 cells were resuspended in 100 \( \mu \)l of PBS, then fixed by addition of 3 ml of 70% methanol. After washing the cells in PBS to remove the methanol, the cells were resuspended in 1 ml of PI staining buffer (50 \( \mu \)g/ml of PI and 15 \( \mu \)g/ml of RNase in PBS). The samples were analyzed using an EPICS Profile II flow cytometer (Coulter Corp., Hialeah, FL). For measurement of annexin V on the cell surface, 1 x 10^6 cells were resuspended in 100 \( \mu \)l of annexin V incubation reagent (TACS Annexin V-FITC, Trevigen, Gaithersburg, MD). The samples were incubated in the dark for 15 min, and then 400 \( \mu \)l of PBS was added. The samples were then analyzed by flow cytometry. Analysis cursors were set based on negative controls and the percentages of labeled cells calculated from events within the limits of the cursors.

**Western blotting.** Total cell lysates were prepared in RIPA buffer with protease inhibitors, and 10 to 30 \( \mu \)g of protein were separated by electrophoresis on 10 to 15% SDS-polyacrylamide gels (depending on the molecular weight of the protein of interest). The proteins were transferred to Hybond-ECL membrane, and the membranes were blocked with 5% milk. The membranes were probed with primary antibodies: anti-p53 (DO-1) mouse monoclonal antibody, anti-p21 (C-19) rabbit polyclonal antibody, anti-bax rabbit monoclonal antibody, and anti-BCL2 mouse monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Secondary antibodies were horseradish peroxidase-conjugated anti-mouse or anti-rabbit antibodies, and the membranes were developed with ECL detection reagents (Amersham, Arlington Heights, IL). To confirm equal loading and transfer of proteins, the filters were stripped and reprobed with rabbit anti-actin antibody (Sigma, St. Louis, MO) and anti-rabbit horseradish peroxidase-conjugated antibody (Amersham). Densitometric calculations of the intensity of bands corresponding to specific proteins were performed using a Personal Densitometer (Molecular Dynamics, Sunnyvale, CA).

**Statistics.** Statistical analyses were performed using the Mann-Whitney test on the GraphPad Prizm program (GraphPad Software, San Diego, CA).

**Results**

**Transduction efficiency following irradiation.** Transduction efficiency was indicated by the percentage of cells expressing \( \beta \)-gal (stained blue). With an MOI of 100, 51% of MDA-MB-231 cells expressed \( \beta \)-gal, while only 15.4% of MDA-MB-435 cells were successfully transduced. When transduction was performed 1 hr after irradiation, the percentages of stained cells increased moderately for both cell lines exposed to the lower MOI values (Figs. 1a,1b), although the proportions of \( \beta \)-gal–expressing MDA-MB-435 were still much lower than those of the MDA-MB-231 cells. Irradiation also extended the duration of expression of \( \beta \)-gal, and on days 7 and 14 of culture, the percentages of \( \beta \)-gal–expressing cells were higher in the irradiated cells than they were in non-irradiated cells exposed to the same MOI of Ad\( \beta \)-gal. Our results confirmed the previous report that MDA-MB-435 cells have low transduction efficiency with adenoviral vectors [20].

**Transduction of breast cancer cells with Ad5CMV-p53 or Ad5CMV-p21.** The effects of adenoviral transduction on cell growth were assessed by counting numbers of viable cells at different times after exposure to virus. Seventy-two hours after infection with Ad5CMV-p53, MDA-MB-231 cells showed growth inhibition that increased with the MOI of virus, with maximal inhibition of 95% with an MOI of 250
Fig. 1. Transduction efficiency of human breast cancer cells exposed to different MOIs of Adβ-gal. Fig. 1a (top) and Fig. 1b (bottom) show the proportions of cells (MDA-MB-231 cells and MDA-MB-435 cells, respectively) expressing the β-galactosidase product following irradiation (0 Gy, 1.44 Gy, 3.7 Gy) 1 hr before viral transduction. The mean percentages of cells expressing β-galactosidase (from two replicate wells) were scored 24 hr after transduction.

Fig. 2. Transduction of cells with Ad5CMV-p53. Breast cancer cells were exposed to Ad5CMV-p53 (MOI range of 5 to 250) or Adβ-gal (MOI 250) in triplicate wells, and surviving cells were counted 72 hr later. The mean cell number of three wells and the SD were plotted. The MDA-MB-231 cells (Fig. 2a, top) showed dose-dependent growth inhibition, while the dose had minimal effect on the MDA-MB-435 cells (Fig. 2b, bottom).

Fig. 3. Transduction with the Ad5CMV-p53-induced p21 expression in MDA-MB-231. MDA-MB-231 western blot analysis depicts increased p21 expression as the MOI of the virus increases.
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(Fig. 2a). The MDA-MB-435 cells exposed to the same MOI of Ad5CMV-p53 showed much less inhibition, with a maximum of 27% inhibition (MOI 250) compared with Ad-β-gal transduced cells (Fig. 2b). When transduced with Ad5CMV-p21, the maximal growth inhibition achieved with MOI 200 was ≤ 40% in both cell lines (data not shown).

Western blot analysis revealed induction of p21 in cells from both cell lines infected with Ad5CMV-p53 (Fig. 3). The level of p21 protein expressed increased with the MOI of virus. No p21 was detected in lysates from control or β-gal–transduced cells. An increase in p53 was not readily discernible as both of the breast cancer cell lines express abundant levels of the protein, and the antibody used does not distinguish wild-type from mutant protein. Thus, p21 expression was used as a surrogate indicator for the presence of the gene and expression of wild-type p53 protein in the transduced cells. Ad5CMV-p21 expression resulted in p21 protein expression in amounts proportional to the MOI of virus. Abundant protein was detected in the lysates of cells sampled 24 hr after transduction with 25 to 200 MOI of virus (data not shown).

As irradiation moderately improved the transduction efficiency of MDA-MB-231 cells with Adβ-gal, exposure to 3.7 Gy was combined with Ad5CMV-p53 and Ad5CMV-p21 transduction. The MOI of 125 was used to allow detection of any changes in cell number and survival time. Cells irradiated 24 hr before transduction with Ad5CMV-p53 had 57% growth inhibition compared with 50% inhibition of non-irradiated cells. When the cultures were irradiated 1 hr before, or 24 hr after, viral transduction, there were no differences in survival of the irradiated cells compared with those of the non-irradiated cells. This is in contrast to other studies that have shown a greater effect when Ad5CMV-p53 is combined with irradiation [26,27], but that may be due to the low MOI of virus used in this study, to fairly low transduction efficiency, or to both.

Flow cytometric assays were used to quantify the proportions of apoptotic cells with and without radiation. When using PI staining 72 hr after transfection on cells irradiated 24 hr before Ad5CMV-p53 transduction, the proportion of cells in the sub-G1 population was higher than it was in cells treated with only virus (Table 1). Annexin V staining is a more sensitive indicator of apoptosis [28-30], and this assay also showed a higher number of apoptotic cells in the samples that were irradiated and then transduced with Ad5CMV-p53, compared with those that underwent Ad5CMV-p53 transduction alone (Fig. 4). For the Annexin V assay, the optimal time to detect apoptotic cells was found to be 48 hr after transduction. Protein analyses showed that irradiation alone induced expression of p21 in the MDA-MB-231 cells, although higher levels of expression were seen in cells exposed to irradiation combined with Ad5CMV-p53 (Fig. 5). As wild-type p53 is reported to regulate box, the levels of box protein were compared. The greatest

<table>
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<th>Treatment</th>
<th>Cell number (% of control)</th>
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*MDA-MB-231 cells were irradiated with 3.7 Gy. Twenty-four hr later, the cells were transduced with AdCMV-p53, Ad5CMV-p21, or Adβ-gal (MOI 125). After incubation for 72 hr, the cells were harvested, counted, and fixed in ethanol for propidium iodide staining. The cell number harvested in each treatment group is expressed as a percentage of cells transduced with the β-gal vector in either the irradiated or non-irradiated group. Ad5CMV-p53 plus irradiation displayed an accumulation in sub-G1 that correlates with apoptosis seen in Annexin studies (See Fig. 4). Although there was an increase in cell number among the cells treated with Ad5CMV-p21 and irradiation, no change is seen in the cell cycle analysis compared with controls. Cells treated with Ad5CMV-p53 and irradiation had greater growth inhibition than ones treated with Ad5CMV-p53 alone compared to β-gal controls.
In contrast to these results, the combination of irradiation and Ad5CMV-p21 resulted in protection from cytotoxicity of the irradiation. While Ad5CMV-p21 transduction alone led to reduced cell numbers, when the cells were irradiated before transduction (either 24 hr or 1 hr), the Ad5CMV-p21-expressing cells had a growth advantage over the control transduced and irradiated cells (Fig. 6a). A clonogenic assay confirmed the protective effect of Ad5CMV-p21 in irradiated cells (Fig. 6b). The sequence of transduction and irradiation was important for p21 to be protective, and no effect was noted in cells irradiated 24 hr after transduction with Ad5CMV-p21 (data not shown).

As noted above, irradiation alone resulted in p21 expression in MDA-MB-231 cells, but not in significant G1 arrest. Irradiation resulted in accumulation of control and β-gal–transduced cells in G2, 24 hr after transduction. Ad5CMV-p21 transduction of irradiated and non-irradiated cells resulted in increased proportions of cells arrested in G1, 24 hr after transduction. This G1 arrest had resolved by 72 hr after transduction. Ad5CMV-p21 transduction did not significantly alter the proportions of dead cells (based on PI or Annexin V assays).

**Combination of adenovirus and chemotherapy.** The loss of functional p53 has been linked with resistance to chemotherapeutic agents and other inducers of apoptosis [31,32]. The sequence of viral transduction with Ad5CMV-p53 and Ad5CMV-p21 followed by chemotherapy was chosen to test whether chemosensitivity was increased by introducing these genes. The results of one clonogenic assay in Fig. 7 showed increased survival rate of drug-treated Ad5CMV-p21 transduced cells (compared with β-gal–transduced controls). In repeat assays, significantly more surviving clones were found only for cells exposed to VP-16 or paclitaxel, with no consistent alteration in the numbers of surviving colonies of doxorubicin-treated cells. In contrast to some other reports, the combination of Ad5CMV-p53 with chemotherapy drugs did not consistently decrease the number of surviving clones.

**Discussion**

Attempting to correct genetic abnormalities through gene therapy is a logical direction for new treatment
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Fig. 5. Western blot analysis of p21 expression in MDA-MB-231 cells with and without irradiation. MDA-MB-231 cells were irradiated with a dose of 0 or 3.7 Gy 1 hr prior to transfection with Ad5CMV-p53 at MOIs of 25 and 125. Cells were harvested and counted on day 5 after transfection, and lysates were made. Western blotting depicts increased p21 expression in irradiated compared to non-irradiated controls. Densitometric ratios of p21 to actin are shown.

Fig. 6. Growth and survival of MDA-MB-231 cells irradiated before adenoviral transduction. Fig. 6a (left) shows increased cell numbers in cultures harvested 4 and 6 days after transduction with Ad5CMV-p21 (MOI 125) compared to β-gal and control. In clonogenic assays (Fig. 6b, right), cells were harvested 24 hr after transduction and replated, and the numbers of surviving colonies counted after 14 days (* = p < 0.05 by t-test, versus controls). Cells treated with Ad5CMV-p21 and irradiation formed more colonies than those with irradiation alone, irradiation + β-gal, or irradiation + Ad5CMV-p53.

Fig. 7. Clonogenic assays for the effect of combining adenoviral transduction and chemotherapy. MDA-MB-231 cells were transduced with adenovirus (MOI = 125) and exposed to the drug 24 hr later. After 24 hr incubation, cells were harvested, replated in 100-mm culture plates, and the numbers of surviving colonies counted at 14 days (* p < 0.05, t-test). Although Ad5CMV-p21 appeared to protect the cells from cytotoxic effects of paclitaxel, etoposide, and doxorubicin, repeated assays showed protection only from paclitaxel and etoposide.
of breast cancers and other cancers. For example, the restoration of wild-type p53 or the introduction of elements such as p16 in the Rb pathway of cell cycle control are two active areas of gene therapy for cancer [13,33,34]. The effectiveness of gene therapy depends in part on the efficiency of gene transfer into the cancer cells. This makes the adenovirus an attractive vector [13,19]. Characteristics of the target cancer cells can also determine rates of gene transfer with adenoviral vectors. As reported by Nielsen et al [20], we found that transduction efficiency with Ad5-β-gal was considerably less in the MDA-MB-435 cell line than it was in the MDA-MB-231 breast cancer cell line. The αv integrins have been implicated in the internalization of adenoviruses after the initial binding to cell surface receptors. The two breast cancer cell lines express similar levels of the αv integrins (Price, unpublished), suggesting that the difference in transduction efficiency is not a function of integrin-mediated internalization but is due to an undefined difference in cell phenotype. Coxsackie virus and adenovirus receptors have been described as common receptors for both virus types [35], but these receptors have not been studied in the breast cancer cell lines. As transduction of MDA-MB-435 cells was so inefficient, we thought to combine gene therapy with irradiation to try to increase the transfer and expression of the adenoviral vectors. Irradiation has been reported to improve plasmid DNA transfection efficiency and to improve adenoviral transduction up to 40-fold in one lung cancer cell line [21,22]. One suggested mechanism is related to the increased DNA activity repair and recombination following irradiation. In our study, irradiation 1 hr before exposure to adenovirus improved the transduction efficiency of the breast cancer cells and extended the duration of expression of β-gal.

In theory, the combination of gene therapy and irradiation could have two advantages. First, the rate of gene transfer into the target cells may be improved. Secondly, there may be synergism or an additive effect that would enhance the therapeutic effect of either treatment modality. The combination of Ad5CMV-p53 and irradiation has been reported to increase rates of cell death in vitro and to increase therapeutic benefit in vivo [26,27]. Our data confirm previous in vitro studies. Higher numbers of apoptotic cells were seen in samples of MDA-MD-231 cells that were irradiated and then transduced with Ad5CMV-p53, compared with samples that were either irradiated or transduced. Increased levels of bax were measured in the Ad5CMV-p53-transduced cells, which is to be expected since bax is a p53-inducible gene [36]. A surprising result was that the Ad5CMV-p53–irradiated and irradiated cells expressed lower amounts of bax than Ad5CMV-p53 transduced cells, yet had higher proportions of apoptotic cells. Presumably, the combination of radiation and p53 expression led to induction of pro-apoptotic genes, in addition to bax. Polyak et al [37] have described a number of other p53-inducible genes that contribute to apoptosis, and one or more of these may be involved in the increased apoptosis following viral transduction and irradiation of the breast cancer cells. Flaman et al [38] have described p53 mutants that induce bax but not p21. These mutants would be used to delineate the role of bax in apoptosis in cells treated with p53 alone and with a combination of radiation and gene therapy.

As noted in previous reports, Ad5CMV-p21 transduction led to cell cycle arrest and growth inhibition of p53-mutated breast cancer cell lines [39]. However, when Ad5CMV-p21 was combined with radiation, the result was protection from the cytotoxic effects of the radiation. The cdk inhibitor p21 is best known for its role in arresting cells in the G1 phase of the cell cycle, following induction by wild-type p53 in response to DNA damage [14,40]. Induction of p21 independent of p53 has also been reported, following irradiation or growth factor stimulation [41,42], through the MAP kinase pathway [43]. The cell cycle arrest caused by p21 is thought to give cells time to repair damaged DNA [44,45]. Experiments with p21−/− cell lines have shown that p21 is necessary for preventing cells with significant DNA damage from progressing into S-phase and may contribute to altering sensitivity to DNA-damaging agents [14,46,47]. Ionizing radiation-induced DNA damage is repaired through recombinational repair pathways [48]. Our results showing protection from cytotoxic effects of ionizing radiation by transduction with Ad5CMV-p21 indicate that overexpression of this gene can alter the consequences of ionizing radiation. Whether this is primarily through inducing G1 arrest and allowing time for repair or through direct involvement in the repair process is not known.
There are conflicting reports in the literature regarding whether p21 expression can protect or sensitize cancer cells from different forms of DNA damaging agents and whether it is involved in apoptosis. Sensitization to cisplatin, radiation, or both by p21 has been reported in studies using human or rodent glioblastoma cell lines [49,50]. In contrast, inducible expression of p21 conferred resistance to BCNU and cisplatin but not to doxorubicin in human glioblastoma cell lines [51]. Human colon cancer cells with p21 deleted by homologous recombination are more sensitive to doxorubicin than the original p21+ cell line [46], while inducible expression of p21 in a different colon cancer line does not alter sensitivity to this drug [17]. In this instance, the difference in outcome may be a function of p53 status, as the first cell line expressed wild-type p53 and the second cell line expressed mutant p53. Overexpression of p21 does not provide significant protection from doxorubicin in the MDA-MB-231 cell line, which has mutant p53. However, the Ad5CMV-p21–transduced MDA-MB-231 cells show greater clonogenic survival after VP-16 or paclitaxel exposure than those of the control-transduced cells. The protection may be a function of the cell cycle, as the cells were exposed when arrested in G1, VP-16 and other antimetabolites have greatest activity against cells in S-phase. Our data suggest that presence of p21 protects against cytotoxicity from some DNA damaging agents but not others, which may suggest a direct role in the different repair processes.

In conclusion, while therapy using p21 as a single intervention can result in cell cycle arrest and restore growth control to cells lacking functional p53, its role in combination with other forms of therapy is questionable. Our data show that p21 expression can give protection from the cytotoxic effects of radiation and of VP-16 or paclitaxel, potentially either by promoting cell cycle arrest or through a role in the repair pathways involved. Therefore, while p21/WAF1/CIP1 can have tumor-suppressing properties [52], Ad5CMV-p21 may not be a promising form of gene therapy when combined with standard forms of therapy. Since p53 induces p21 protein expression, treatment with p53 in combination with other treatment modalities may also be of concern. The positive and negative effects of combination therapies must be studied further to assess their usefulness in clinical applications.

References


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