Bypass of Abnormal MDM2 Inhibition of p53-dependent Growth Suppression

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ABSTRACT

Oncoprotein MDM2 inhibits p53-dependent cell cycle arrest and apoptosis. MDM2-overexpressing human cancer cell lines (n = 3) were found to be resistant to growth inhibition after infection by p53-expressing adenovirus (Ad-p53), as compared to low MDM2-expressing tumors (n = 3), in vitro. The growth of MDM2-overexpressing tumors, however, was inhibited by p21-expressing adenovirus (Ad-p21) infection, and the cyclin-dependent kinase-inhibitory region of p21 was sufficient to bypass the MDM2-p53 feedback loop. The phosphorylation state of Rb correlated with the response to either p53 or p21 gene therapy. MDM2-overexpressing cancer cells infected by Ad-p21 also developed a quiescent large-cell morphology. The results suggest that MDM2-mediated resistance to p53 may be bypassed by p21 and that the Rb phosphorylation state may predict the effects on growth after Ad-p53 or Ad-p21 infection.

INTRODUCTION

Because p53 is a potent growth inhibitor that can induce growth arrest or apoptosis, it shows promise as a target for gene replacement therapy in cancer (1). Much effort has recently focused on the potential use of p53 for the control of cancer cell proliferation both in vitro and in vivo (2-4). We found previously that the effects of Ad3-p53 therapy were influenced by infectivity, p53 mutational status, and the presence of human papilloma virus E6 protein (3, 5). These observations suggested that certain human cancers may not be ideally suited for Ad-p53 gene therapy and that it may be important to identify such cancers and to investigate other strategies for the control of their proliferation.

One potential mechanism for resistance to Ad-p53 infection in human cancer may involve interactions between p53 and the human homologue of the MDM2 oncoprotein. Originally cloned from a spontaneously transformed BALB/c mouse cell line (6), the MDM2 gene is a target for transcriptional activation by p53 (7). The MDM2 protein can bind to p53, but in forming this complex, MDM2 conceals the transactivation domain of p53 and can inhibit transcriptional activation by p53 (7, 8). MDM2 has been shown to inhibit p53-dependent cell cycle arrest and apoptosis (9, 10). In normal cells, the binding of MDM2 to p53 has been hypothesized to form a negative feedback loop in which the p53-activated MDM2 then inhibits p53 function (11). Targeted disruption of the MDM2 gene led to embryonic lethality, which was rescued by simultaneous knockout of the p53 gene (12, 13).

A significant number of human cancers have elevated levels of MDM2, resulting either from gene amplification or from alternate mechanisms, such as enhanced translation (14). Overexpression of MDM2 has been observed in more than 30% of human sarcomas (15), as well as in breast cancers (16), malignant gliomas (17), leukemias (18), lymphomas (19), esophageal carcinomas (20), and melanomas (14). Most of the tumors that possess amplified MDM2 express wild-type p53 protein, which is transcriptionally deficient (21). Although p53 inhibition seems to account for some of the biological effects of MDM2, some evidence suggests that MDM2 may also have p53-independent effects on growth regulation. Overexpression of the mdm-2 gene transforms immortalized fibroblasts alone (22) and in primary fibroblasts in cooperation with activated ras (23). MDM2 has been shown to interact with the retinoblastoma (Rb) protein, which regulates the restriction point through interaction with the E2F family of transcription factors (24, 25). MDM2 mutants that do not bind p53 have been identified in human tumors (26). Recently, MDM2 has been shown to play a p53-independent role in the regulation of DNA synthesis (27).

Because MDM2 is a potent inhibitor of p53-dependent G1 arrest and apoptosis and because MDM2 is overexpressed in many human cancers, we examined whether tumors with elevated levels of MDM2 may be resistant to Ad-p53 gene therapy. After infection with Ad-p53 in cell lines that either have low or high expression levels of MDM2 protein, we analyzed the subsequent induction of MDM2, the phosphorylation state of Rb, the progression of the cell cycle, and the viability of the infected cells. In melanoma and choroid carcinoma cells that overexpress MDM2, Ad-p53 did not cause significant growth inhibition as compared to those cell lines with low levels of MDM2.

We examined the effect of an Ad expressing the cell cycle
and growth inhibitor p21\textsuperscript{WAF1/CIP1} (Ad-p21) on the proliferation of tumors with high MDM2 expression levels. The CDK inhibitor p21\textsuperscript{WAF1/CIP1} is a negative-regulator of the cell cycle after DNA damage or various cellular differentiation signals (28). Use of Ad-p21 has been shown to inhibit cancer cell growth both \textit{in vitro} and \textit{in vivo} (5, 29, 30). In the present study, we examined the potential utility of Ad-p21 to inhibit the growth of cancer cells that overexpress MDM2 and are resistant to Ad-p53. Various molecular markers were examined for their ability to retrospectively predict the susceptibility of cancer cells to growth inhibition by Ad-p53 or by Ad-p21. We found that high endogenous MDM2 levels before infection and persistent Rb hyperphosphorylation after infection correlated negatively with cancer cell growth inhibition by Ad-p53.

**MATERIALS AND METHODS**

**Tumor Cell Lines and Culture Conditions.** The human melanoma cell lines A875 and 7336, the human choriocarcinoma cell line JEG3, and the UMUC3 human bladder cancer cell line, which was obtained from Drs. T. McGarvey and B. Malkowicz (University of Pennsylvania), were maintained in MEM supplemented with Earle’s salts, 1-glutamine, 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin. The human colon carcinoma cell line HCT116, obtained from Dr. B. Vogelstein (Johns Hopkins University, Baltimore, MD), was maintained in McCoy’s 5A media with 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin. The human lung cancer cell line H460, obtained from Dr. S. B. Baylin (Johns Hopkins University), was maintained in RPMI 1640 supplemented with 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin.

**Ad Infections.** Replication-deficient Ad recombinants expressing β-galactosidase (Ad-LacZ) or wild-type p53 (Ad-p53) were generous gifts from Dr. B. Vogelstein (Johns Hopkins University) and were prepared as described previously (31). Ad recombinants encoding wild-type human p21\textsuperscript{WAF1/CIP1} (Ad-p21) and a COOH-terminal deletion mutant of p21\textsuperscript{WAF1/CIP1} that does not interact with PCNA (Ad-p21-341) were prepared as described previously (5, 32). The viral titers of the Ad recombinants were determined by a plaque-forming assay after infection of 293 cells, as described previously (31). All infections were performed in Dulbecco’s PBS without Ca\textsuperscript{2+} or Mg\textsuperscript{2+} but were supplemented with 1% FBS. The infectivity of the cell lines was compared by infecting the cells with the Ad-LacZ recombinant and then staining at 20 h later with 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside, as described previously (3). The MOI was defined as the ratio of the total number of plaque-forming units used in a particular infection per total number of tumor cells to be infected. Control infections consisted of Ad-LacZ and mock procedures in which the cells were only incubated with PBS containing 1% FBS.

**Western Blot Analysis.** At 20 h after infection of 2 × 10\textsuperscript{6} tumor cells with the adenoviral recombinants, total cellular proteins were harvested as described previously (3). A total of 75–100 μg of protein per lane was loaded and electrophoresed through either a 15% (for p53 or p21 analysis) or a 7.5% (for MDM2 or Rb analysis) denaturing polyacrylamide gel and then electroblotted, as described previously (33). Immunodetection of p21 expression was performed using mouse anti-human WAF1 monoclonal antibody EA10 (Oncogene Science), and immunoblotting for p53 expression was performed with anti-human p53 monoclonal pAb1801 (Oncogene Science), as described previously (33). The expression levels of MDM2 were detected with mouse anti-human MDM2 monoclonal antibody IF2 (Oncogene Science). Immunoblotting for Rb expression was performed with an anti-human Rb monoclonal antibody LM95.1 (Oncogene Science).

**Cell Cycle Arrest.** At 20 h after Ad infection of tumor cells, 10 μCi of [\textsuperscript{3}H]thymidine was added to 100,000 cells/well in 24-well plates. The plates were then incubated for 6 h at 37°C, and the incorporation of [\textsuperscript{3}H]thymidine into newly synthesized DNA was determined, as described previously (33).

**Analysis of Cell Viability.** The exclusion of the dye trypan blue was used to assess cell membrane integrity under bright-field microscopy, as described previously (33). Briefly, at 2 days after infection, the number of viable cells was determined in both the floating and adherent cell populations. Long-term cell viability beyond 6 days after infection was evaluated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay (3).

**RESULTS**

**Analysis of MDM2 Expression in Human Cancer Cells.** To examine the role of MDM2 as a potential barrier to successful p53 gene therapy, we examined the levels of endogenous MDM2 in six human cancer cell lines. Melanoma and choriocarcinoma cells were chosen because they overexpress MDM2 protein (14, 34, 35). Fig. 1B shows the relative levels of the endogenous M\textsubscript{R}, 90,000 MDM2 protein in the panel of cell lines. Three cell lines (HCT116 colon, H460 lung carcinoma, and UMUC3 bladder cancer; Fig. 1B, Lanes 1–3) expressed low or undetectable levels of MDM2, whereas three cell lines (A875 and 7336 melanoma and JEG3 choriocarcinoma; Fig. 1B, Lanes 4–6) expressed high levels of MDM2. The latter cell lines contain wild-type p53 and overexpress MDM2 protein via a mechanism of enhanced translation (35).

Fig. 1C compares the infectivity of the different cell lines to Ad (×320). At an MOI of 100, the majority of cells expressed β-galactosidase protein at 20 h after Ad-LacZ infection. The ability of Ad to infect the cell lines used in this study was not dependent on p53 status nor on the expression levels of MDM2. The JEG3 cell line appeared somewhat more resistant to Ad infection than the other cell lines.

**Human Cancer Cells That Overexpress MDM2 Are Resistant to the Growth-Inhibitory Effects Induced by Ad-p53 Infection.** MDM2 is a potent inhibitor of p53-dependent cell cycle arrest and apoptosis (9). We examined our panel of six cancer cell lines to determine whether the expression levels of MDM2 could be correlated with a resistance to cell cycle arrest after Ad-p53 infection. Fig. 2 shows that the three cancer cell lines with low or undetectable endogenous MDM2 protein levels (HCT116 colon cancer, H460 lung cancer, and UMUC3 bladder cancer) were significantly arrested after Ad-p53 infection at both an MOI of 20 (Fig. 2A) and 100 (Fig. 2B). In all of the cell lines tested, infection with Ad-LacZ at an MOI of 20 or 100 did not significantly arrest DNA synthesis compared to the mock-infected cells. Some toxicity, however, with Ad-LacZ at
Fig. 1  MDM2 expression levels and infectivity of a panel of human cancer cells.  

A, outline of experimental scheme. All cell lines were infected with the Ad recombinants at day 0, and DNA synthesis and protein expression were analyzed 20 h later. Short-term cell viability was assessed on day 2, and long-term viability was examined beyond day 6, as described in “Materials and Methods.” In B, Western blot analysis of the endogenous MDM2 expression level of cancer cells was performed as described in “Materials and Methods.” In C, the infectivity of the cell lines was assessed with 5-bromo-4-chloro-3-indolyb-3-D-galactopyranoside staining at 20 h after Ad-LacZ infection at an MOI of 100, as described in “Materials and Methods” (×320).

an MOI of 100 was apparent when compared to the mock-infected cells (Fig. 2, A and 2B).

Cells that overexpressed MDM2 (the melanoma cell lines A875 and 7336 and the choriocarcinoma JEG3) were resistant to the cell cycle inhibitory effects of Ad-p53 (MOI of 20) at 20 h after infection (Fig. 2A). After Ad-p53 infection, the incorporation of $[^3H]$thymidine in tumors overexpressing MDM2 did not significantly differ from LacZ- or mock-infected cells. Although DNA synthesis was decreased slightly in two cell lines with elevated MDM2 levels following Ad-p53 infection at an MOI of 100, the growth arrest was not as significant as that induced by Ad-p53 in tumors with low endogenous MDM2. Infection with Ad-p53 almost completely suppressed DNA synthesis in the HCT116, H460, and UMUC3 tumor cells at either an MOI of 20 or of 100 (Fig. 2B). These results suggest that high MDM2 expression may decrease the effectiveness of p53 gene therapy.

Ad-p21 Can Bypass the p53 Resistance Observed in MDM2-overexpressing Cancer Cells. We showed previously that p21 is a potent growth inhibitor of human cancer cells that express the human papillomavirus E6 protein (5). We investigated whether Ad-p21 could similarly bypass the MDM2-p53 negative feedback loop. Ad-p21 infection of A875 and 7336 melanoma cells, which were resistant to Ad-p53, now resulted in a strong inhibition of cell cycle progression, similar
to the cell lines that do not overexpress MDM2 (Fig. 2). The MDM2-overexpressing tumor cells were equally susceptible to Ad-p21-341, an Ad construct that expresses the cyclin-CDK-inhibitory domain of p21, which is sufficient for tumor growth inhibition (Fig. 2). In the present study, the inhibition of DNA synthesis was observed at an MOI of 20 after both Ad-p21 or Ad-p21-341 infection (Fig. 2A). Although the tumors that overexpress MDM2 had been almost completely resistant to the growth-arresting properties of Ad-p53 (Fig. 2), infection with Ad-p21 or Ad-p21-341 at an MOI of 100 almost completely suppressed DNA synthesis (Fig. 2B). The results suggest that Ad-p21 may be useful in circumventing the resistance of such MDM2-overexpressing melanoma and choriocarcinoma cells to growth inhibition by Ad-p53. The results suggest that the cyclin-CDK-interacting domain of p21 is sufficient for bypassing p53 resistance in MDM2-overexpressing cancer cells.

The Basal MDM2 Protein Level Rather Than the Exogenous p53-Inducible Level of MDM2 Correlates with the Resistance of Tumors to Infection by Ad-p53. To further study the molecular mechanisms underlying the resistance of MDM2-overexpressing cancer cells to Ad-p53, the protein expression levels of p53 (Fig. 3A), p21 (Fig. 3B), and MDM2 (Fig. 3C) were determined after Ad infection at an MOI of 20. Western immunoblotting confirmed the presence of greatly increased p53 protein levels after Ad-p53 infection, whereas infection with Ad-LacZ or Ad-p21 produced no such overexpression of p53 (Fig. 3A). The levels of endogenous and exogenous p53 protein in MDM2-overexpressing melanoma cells were then compared with the levels in cells that had low levels of MDM2 and that were sensitive to Ad-p53. No differences in p53 expression were found between cells expressing low or high levels of MDM2. JEG3 cells, however, did not express equally high levels of exogenous p53, possibly because of its slightly lower infectivity.

As expected, significant p21 overexpression was observed following infection with Ad-p21 but not with Ad-LacZ or Ad-p53 (Fig. 3B). The endogenous levels of p21 were increased to a greater extent after Ad-p53 infection in tumors with low MDM2 (HCT116, H460, and UMUC3) than tumors with high MDM2 levels (A875, 7336, and JEG3). Thus, cell lines that overexpress MDM2 displayed a blunted transcriptional response in terms of p21 induction, which correlated with increased resistance to Ad-p53. These findings were confirmed by immunofluorescent staining for p21 protein expression (data not shown). Infection with Ad-p21, however, consistently produced higher levels of p21 protein compared to Ad-p53, regardless of the status of MDM2 in the cancer cells (Fig. 3B). These results suggest that a high induction of p21 after Ad-p53 infection may correlate with a more pronounced cellular sensitivity to the growth-suppressive effects of Ad-p53.

A similar correlation between protein induction after Ad-p53 infection and degree of sensitivity to Ad-p53 was also observed for MDM2 expression in the cell lines (Fig. 3C). Tumors that normally have low or undetectable endogenous MDM2 (HCT116, H460, and UMUC3) expressed a significantly enhanced induction of MDM2 after Ad-p53 infection. Infection with Ad-LacZ or Ad-p21 did not induce MDM2 to similar levels. In contrast, the melanoma cells, which have a high basal expression of MDM2, had a blunted induction of MDM2 protein after Ad-p53 infection. Yet, despite the greater induction of MDM2 mediated by Ad-p53 in tumors with normally low basal levels of MDM2 (HCT116, H460, and UMUC3), the melanoma cells were still extremely susceptible to the growth inhibition caused by Ad-p53. In the melanoma cells,
Basal expression of MDM2 rather than the p53-inducible expression of MDM2 correlates with the resistance of tumors to infection by Ad-p53. A, Western blot analysis of p53 expression after Ad infections (as indicated) at an MOI of 20 in low (HCT116, H460, and UMUC3) or high (A875, 7336, and JEG3) MDM2-expressing human cancer cells. B, Western blot analysis of p21 expression after Ad infections (as indicated) in low or high MDM2-expressing human cancer cells. C, Western blot analysis of MDM2 expression after Ad infections (as indicated) in low or high MDM2-expressing human cancer cells. Infections and Western analyses were performed as indicated in Fig. lA and described in “Materials and Methods.”

However, although endogenous MDM2 was overexpressed, cell cycle arrest remained poor despite the apparently lower total levels of MDM2 relative to p53 after Ad-p53 infection (Fig. 3, compare A and C). The results suggest that low endogenous levels of MDM2 protein may be correlated with an enhanced susceptibility to growth inhibition after Ad-p53 infection.

Persistence of the Hyperphosphorylated Form of Rb Correlates with Tumor Resistance to Ad-p53 Infection. Because Rb is a critical regulator of the G1-to-S-phase transition in mammalian cells (36) and because both p53-deficient and Rb-deficient cells have defective checkpoint control (37, 38), we examined the phosphorylation state of Rb as a potential indicator of response to Ad-p53 infection. In addition, because MDM2-overexpressing cells (A875 and 7336) displayed a blunted p21 transcriptional response after Ad-p53 infection (Fig. 3B), we hypothesized that in these cells, Rb might remain in a hyperphosphorylated state. Persistence of the hyperphosphorylated state of Rb would permit cells to progress through the cell cycle, preventing Ad-p53-induced growth arrest. Western analysis of HCT116, H460, and UMUC3 tumor cells that were sensitive to the growth-suppressive effects of Ad-p53 showed that the predominant form of Rb that existed after Ad-p53 infection was the hypophosphorylated form (Fig. 4). The Ad-p53-resistant cell lines, however, had hyperphosphorylated Rb, which was also observed after mock and Ad-LacZ infections. In contrast to Ad-p53, infections with Ad-p21 or Ad-p21-341 resulted in efficient hypophosphorylation of Rb in all of the cell lines, which correlated with an inhibition of cell cycle progression. JEG3 cells, which were not efficiently growth suppressed by Ad-p21, also had a persistent hyperphosphorylated Rb. These results suggest that the phosphorylation state of Rb may correlate with the degree of growth inhibition after Ad-p53 or Ad-p21 infection. Interestingly, the fact that hypophosphorylated Rb in MDM2-overexpressing cells correlated with cell cycle arrest after Ad-p21 infection suggested that cell cycle deregulation mediated by an interaction between MDM2 and Rb (25) could be overcome by p21 overexpression.

Decreased Cell Viability, Decreased Growth, and Altered Cellular Morphology in MDM2-overexpressing Cells after Infection with Ad-p21 but not with Ad-p53. We examined cell viability on a short-term (2 days) and on a longer-term basis (greater than 6 days) after Ad infection. At 2 days after infection, a greater number of nonviable floating cells was observed in the tumors with low endogenous MDM2 (HCT116, H460, and UMUC3), which were more susceptible to Ad-p53 (Fig. 5A). In these same cell lines, the number of adherent cells was significantly decreased after Ad-p53 or Ad-p21 infection but not after either mock or Ad-LacZ infection (Fig. 5A).
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decrease in cell number after Ad-p21 infection further suggests over time, as compared with Ad-p53 infection. Fig. SB shows the total cell population was observed after Ad-p53 infection hyperphosphorylated (long arrows) decrease in cell viability was observed after either Ad-p53 or Ad-p21 infection. In the low MDM2-expressing cell line, UMUC3, a noticeable that Ad-p21 could bypass an MDM2-mediated resistance to p53. Alternatively, it is possible that the ratio of MDM2 to p53 remains low enough to permit low MDM2-expressing tumors to remain sensitive to p53. It is also possible that such tumors may remain sensitive to p53 through cell death mechanisms that are not dependent on inhibition of the transcription function of p53 by MDM2. It is conceivable that high MDM2-expressing tumors may be resistant to transcription-independent apoptosis, as well as to transcription-dependent apoptosis. Although MDM2 overexpression in the three cell lines used in this study was based on enhanced translation, no evidence presently suggests that the mechanism of MDM2 overexpression may influence its function.

DISCUSSION

MDM2-overexpressing tumors represent a significant fraction of various tumor types, and it appears that such tumors would not be adequately treated with Ad-p53. It is not entirely clear why the extremely high levels of MDM2 induced by exogenous p53 were not sufficient to suppress p53-dependent cell cycle inhibition or apoptosis in the low (endogenous) MDM2-expressing cancer cells. We speculate that cell cycle inhibition was resistant, perhaps as an early event, prior to the transcriptional activation of MDM2 by p53. However, the effects on cell viability, which were determined over the first 10 days after infection, are more difficult to explain. One possibility is that there may be a biochemical difference between endogenous and newly synthesized MDM2 proteins, so that the newly synthesized MDM2 is not an effective inhibitor of p53. Alternatively, it is possible that the ratio of MDM2 to p53 remains low enough to permit low MDM2-expressing tumors to remain sensitive to p53. It is also possible that such tumors may remain sensitive to p53 through cell death mechanisms that are not dependent on inhibition of the transcription function of p53 by MDM2. It is conceivable that high MDM2-expressing tumors may be resistant to transcription-independent apoptosis, as well as to transcription-dependent apoptosis. Although MDM2 overexpression in the three cell lines used in this study was based on enhanced translation, no evidence presently suggests that the mechanism of MDM2 overexpression may influence its function.

The bypass of p53 resistance in MDM2-overexpressing cancer cells through the use of p21-expressing Ad has some interesting implications. It appears that the phosphorylation state of Rb may be a useful indicator of cancer cell sensitivity to either p21 or p53. High MDM2-expressing melanoma cells hypophosphorylated Rb after Ad-p21 but not Ad-p53 infection. The results suggest that high levels of endogenous MDM2 were

![Fig. 4 Persistence of the hyperphosphorylated form of Rb correlates with cancer cell resistance to Ad-p53 infection. Western blot analysis of hyperphosphorylated (long arrows) and hypophosphorylated (short arrows) forms of Rb after Ad infections (as indicated above each lane) of low (HCT116, H460, and UMUC3) or high (A875, 7336, and JEG3) MDM2-expressing cancer cells. Infections and Western blot analyses were performed as indicated in Fig. 1A and described in "Materials and Methods."](http://clincancerres.aacrjournals.org/)
Fig. 5  Decreased cell viability, decreased growth, and altered cellular morphology in MDM2-overexpressing cells after infection with Ad-p21 but not with Ad-p53. A, analysis of cell viability 2 days after Ad infections (as indicated below each lane) of low (HCT116, H460, and UMUC3) or high (A875, 7336, and JEG3) MDM2-expressing cancer cells. Control cell viability was determined at the time of infection (day 0). Viable (●) and nonviable (■) cell counts were determined for floating (left panels), adherent (middle panels), or the total cell population (right panels) as indicated in Fig. 1A and described in “Materials and Methods.” B, long-term viability of low MDM2-expressing UMUC3 bladder cancer cells (upper panel) or of high MDM2-expressing A875 cells was determined with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay on day 6 (UMUC3 cells) or day 10 (A875 cells) after infections with various Ads (as indicated below each column). C, phase microscopic examination (low power view) of high MDM2-expressing A875 melanoma cells at 6 days following Ad-LacZ, Ad-p53, Ad-p21, or Ad-p21-341 infection (X100). Arrows, multinucleated giant cells containing large cytoplasmic vacuoles. Infections were performed at an MOI of 100 as described in “Materials and Methods.”
not capable of overcoming p21-mediated growth arrest. These results were somewhat surprising in view of previous results demonstrating that MDM2 can interact with Rb and permit E2F to facilitate S-phase progression (25). We ruled out the possibility that wild-type p21 may be having its effects through interaction with PCNA (at a step downstream of Rb) because we observed identical results using Ad that expresses p21 lacking its PCNA-interacting domain. Thus, the cyclin-CDK-interacting domain of p21 was sufficient to inhibit the proliferation of MDM2-overexpressing cancer cells. p21-expressing Ad may be useful for bypassing resistance to p53 in such tumors. In JEG3, however, growth inhibition was not as efficient as in the malignoma cell line, but JEG3 was also poorly infected by Ad, as demonstrated by Ad-LacZ. These cells still failed to hypophosphorylate Rb, suggesting that its persistence may be a marker for infectivity as well. The nature of the p21 growth-arrested giant cells and the role of Rb in the formation of these giant cells need to be further investigated.

The results of the present study demonstrate that gene therapy using wild-type p53 may not effectively suppress the growth of tumors that overexpress the MDM2 oncoprotein. A potential alternative may be the use of an Ad vector that expresses p21, a potent growth inhibitor that bypasses the p53-MDM2 feedback loop. Other alternatives include gene replacement using a mutant p53 that is incapable of interaction with MDM2 (9), drugs that block the p53-MDM2 interaction (41), or perhaps the use of E1B-deleted Ad (42). The data further suggest that the phosphorylation state of Rb may be a useful indicator of cancer cell sensitivity to Ad-p53 or to Ad-p21 therapy in vitro and that its utility should be further investigated in vivo.

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REFERENCES

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