Efficient Induction of Cell Death by Adenoviruses Requires Binding of E1B55k and p53

Brett R. Dix, Simon J. O’Carroll, Colleen J. Myers, Sara J. Edwards, and Antony W. Braithwaite

Cell Transformation Group, Department of Pathology, Dunedin School of Medicine, Dunedin, New Zealand 9001

ABSTRACT

The use of an E1b55k-deficient adenovirus, ONYX-015, to selectively target tumor cells containing a mutated p53 gene has produced promising results. However, recent reports have questioned the selectivity of this virus, showing that ONYX-015 can replicate in cells containing a wild-type p53 and that p53 may actually be required for cell death. To address these apparent contradictions in the literature, we infected a number of mutant and wild-type p53-containing cell lines with ONYX-015 and wild-type adenovirus and observed their death profiles up to 10 days postinfection. We demonstrate that two distinct cell death phenotypes exist, one of which is rapid and dependent on the presence of p53 and one of which is p53 independent. Using adenoviruses expressing E1b55k proteins deficient in their ability to bind p53, we show that formation of a complex between p53 and the adenoviral E1b55k protein is necessary for the activation of the rapid cell death pathway. In the absence of p53 or the absence of complex formation between p53 and E1b55k, cell death is delayed considerably. These data suggest three things: that the selectivity of killing appears to be dependent on the presence of the E1b55k/p53 complex; that viruses lacking E1b55k (such as ONYX-015) kill cells in a delayed manner independent of p53; and that binding of E1b55k to p53 does not merely serve to inactivate p53, but rather is required for the induction of rapid cell death. The components of this complex that lead to rapid cell death remain to be determined.

INTRODUCTION

An attenuated adenovirus ONYX-015 (dl 1520), deficient for expression of the early E1b55k protein, is currently undergoing clinical trials for the treatment of head and neck cancers, with apparently promising results (1). This virus is thought to be useful therapeutically because of its ability to replicate selectively in tumor cells deficient in p53 function but not in cells containing a normal p53 (2, 3). However, recent reports have indicated that both wtAd5 and ONYX-015 replicate in all cells tested irrespective of their p53 status (4–6). Moreover, these results demonstrated that wtAd5, almost without exception, replicated more efficiently than ONYX-015. Such data are generally inconsistent with the proposed model for selective destruction of tumor cells by ONYX-015.

In addition to these findings, our laboratory reported that cell death induced by wtAd5 was dependent on the presence of a wt p53 protein (4). However, others reported that the induction of cell death by wtAd5 was independent of p53 status (5), which is seemingly at odds with our findings. However, examination of these results indicates that the kinetics of cell death in the presence or absence of p53 are different. Cells expressing a wt p53 died more quickly than cells defective in p53 (5). Such results suggest there may be multiple cell death processes: one requiring p53, which kills cells rapidly, which we have reported previously (4), and at least one other, independent of p53, which kills more slowly. If true, this may explain the differences between our data and those of others and also explain how ONYX-015 can destroy tumor cells deficient in p53, although it does not explain its selectivity for tumor cells.

In this study, we explored this possibility by determining the kill rates of wtAd5 and ONYX-015 in a panel of human cell lines that differ in their p53 status. We show that cells expressing a wt p53 die more rapidly than either cells with a mutant p53 or cells that are null for p53, after infection with wtAd5. These data confirm the existence of kinetically distinct cell death processes. We also show that the rapid p53-dependent death is markedly delayed or absent after ONYX-015 infection, suggesting that E1b55k is also necessary for this cell death. Because E1b55k binds p53, we hypothesized that formation of this complex may be necessary for induction of this rapid death pathway. We confirmed this hypothesis using a panel of E1b55k mutant viruses, which contain mutations that disrupt binding to p53. Thus, the widely held view that binding of E1b55k to p53 abrogates p53 function is not correct in the context of viral infection.

MATERIALS AND METHODS

Cell Lines. Ten human cell lines were used for the studies described in this paper. 293 cells (an embryonic kidney cell line containing the E1a and E1b genes) were used for propagating wtAd5 and mutant viruses (7). The cell lines containing wt p53 were A549 lung cancer cells (8), HepG2 hepatoma (9), HT 1080 fibrosarcoma (10), RKO colon carcinoma (11), and primary HFS. The cell line expressing a mutant p53 was T98G glioma, which contains an M-237-1 mutation (12). p53-null cell lines were IICF/c skin fibroblasts derived from a Li Fraumeni patient (13) and Saos 2 osteosarcoma (14). RKO p53.13 cells are RKO cells transfed with a dominant negative mouse p53 mutant (11). All cell lines were grown in DMEM (Life Technologies, Inc.) supplemented with 10% FBS at 37°C and 10% CO2.

Adenoviruses. WtAd5 and mutants ONYX-015 (dl 1520), dl 338, F484, R443, H354, R309, R180, A262, and H326 were used for the experiments described in this paper. These viruses have been described previously, and the appropriate references are shown below. Briefly, these viruses consist of the following mutants. ONYX-015 (dl 1520; Ref. 15) and dl 338 (16) do not produce detectable E1b55k products. F484, R443, H354, R309, R180, A262, and H326 viruses contain 12-bp insertion mutations immediately following the codons indicated by the mutant numbers, and all express stable E1b55k proteins (17). All viruses were grown on 293 cells and titrated on 293 cells using a CPE assay. Virus titers were standardized in CPEUs, the details of which have been described elsewhere (18). CPEUs are approximately equivalent to plaque-forming units.

Cell Viability Assay. Cells (3 × 10^4) were seeded into each well of six-well tissue culture plates and incubated for 3–4 h in DMEM + 10% FBS. After this, cells were mock infected or infected with 10 CPEUs/cell of adenovirus and further incubated in DMEM + 10% FBS. At indicated times postinfection, both floating and trypsinized cells were harvested and suspended in their existing medium. An equal volume of PBS containing 0.1% trypsin blue was added to the cell suspension. Viable and nonviable cells were then determined by direct counting. All counts were performed on triplicate samples, and means were plotted on all graphs, with SEs shown when greater than 5% of the mean.

Cell Synchronization. Cells (4 × 10^5) were seeded into six-well tissue culture plates and incubated for 3–4 h in DMEM + 10% FBS. Medium was...
replaced with DMEM + 10% FBS containing 1.5 mM hydroxyurea and incubated for a further 16 h. Medium was removed, cells were washed twice with PBS, and replaced with DMEM + 10% FBS. Cells were incubated for a further 5 h before being mock infected or infected with 10 CPEUs/cell of adenovirus. At indicated times postinfection, cells were harvested, and viable and nonviable cells determined by trypan blue staining and direct counting. All counts were performed in triplicate and plotted as means, with SE shown if greater than 5% of the mean.

FACS Analysis. Cells were harvested, centrifuged at 400 × g for 10 min, and resuspended in sample buffer (PBS containing 1 g/liter glucose). Cells were washed twice more in sample buffer and fixed in 70% ethanol by incubation at 4°C for at least 12 h. Fixed cells were resuspended in a solution of 50 μg/ml propidium iodide in sample buffer containing 2 mg/ml RNase A and incubated at room temperature for at least 30 min. Samples were analyzed within 2 h on a Becton Dickinson FACScalibur analyzer. All samples from individual experiments were analyzed at the same time, and 0-h, uninfected controls were used to calibrate the FACS analyzer settings prior to reading the remaining samples. Cells were gated as demonstrated in Fig. 3 using FL2 area versus FL2 width to exclude doublets and cell aggregates.

RESULTS

Two Distinct Cell Death Phenotypes Are Evident after Infection with Adenoviruses. To determine whether adenovirus induces more than one type of cell death, at least one that is p53 dependent and one that is independent of p53, a panel of human tumor cell lines differing in their p53 status was infected with wtAd5. Cell death determinations were made out to 10 days postinfection, which is approximately 6 days longer than in our previous experiments (4) and is similar to those reported elsewhere (2, 3, 5). The results of these experiments are shown in Fig. 1. A549, HepG2, and HT 1080 cells, which express a wt p53, underwent rapid cell death, with less than 30% of cells remaining viable by day 4 (Fig. 1, a–c). These results are similar to our previous findings (4). However, T98G cells, which express a mutant p53 protein, and IICF/c and Saos 2 cells, which do not express p53, retained cell viabilities of 70–90% by day 4 after infection with wtAd5 (Fig. 1, d–f). Death did eventually occur in T98G and IICF/c cells, but this was delayed considerably (Fig. 1, d and e). In Saos 2 cells, death was not delayed as extensively as in the other p53-deficient cell lines but still showed a 2–3-day lag time (Fig. 1f). To address whether these different death profiles might be cell type-specific events, we performed an identical assay on RKO and RKO p53.13 cell lines. RKO p53.13 cells arose by stable transfection of a dominant negative mouse p53 into the RKO parental cell line thereby eliminating p53 function (11). Whereas RKO cells demonstrated rapid death similar to that induced in the wt p53-containing cell lines, RKO p53.13 cells showed a delayed death profile similar in kinetics to the other p53-deficient cell lines (Fig. 1, g and h). These data demonstrate the presence of two different death phenotypes based upon the presence or absence of a wt p53 protein.

When the same panel of cells was infected with the E1b55k-deficient ONYX-015, we observed delayed cell death in A549 and HepG2 cells with kinetics similar to those of wtAd5 infection of mutant p53 cells (Fig. 1, a and b). In all other cells examined, ONYX-015 failed to induce cell death to a greater extent than uninfected controls. Where there is overlap of cell lines (A549 and Saos 2), our results are essentially identical for both wtAd5 and ONYX-015 to those reported previously (5). These data show that E1b55k promotes death, because wtAd5 kills more quickly than ONYX-015 in either the presence or absence of p53. This suggests that E1b55k may play an important role in enhancing the p53-dependent and p53-independent cell death phenotypes.

To determine whether these data were truly reflective of infection of normal human cells, and not an artifact of cell immortalization, similar experiments were carried out in cultures of HFs. To achieve the same extent of cell killing, a 30-fold greater titer of virus was required (i.e., 300 CPEUs/cell), but again wtAd5 caused cell death much faster than ONYX-015 (Fig. 1f). Less efficient infection of “normal” cells has been reported recently for a number of early passage human cells (6).

These data lead us to conclude that adenovirus induces more than one cell death pathway that is dependent on the presence of E1b55k and p53.

E1b55k Relieves Growth Arrest. In the course of carrying out the above experiments, we noticed that in addition to remaining viable for much longer than their counterparts infected with wtAd5 (see Fig. 1), fewer cells remained after ONYX-015 infection. This suggested that ONYX-015 might be slowing or inhibiting cell division. To test this directly, A549 cells were infected with wtAd5 and with two E1b55k mutants (dl 338 and ONYX-015). At different times after infection, total cell number was determined, and cell cycle analyses were carried out by flow cytometry. Results demonstrate that cells infected with wtAd5 (Fig. 2a) died within 4 days, as demonstrated previously (Fig. 1a; Ref. 4). However, cells infected with the two E1b55k-deficient viruses failed to proliferate (Fig. 2, b and c), and there was no decline in viability (as shown in Fig. 1) over the time course of the experiment.

To examine these phenotypes in an alternative way, flow cytometry was performed. This analysis (Fig. 3) showed that by 48 h postinfection, cells infected with wtAd5 exhibited a decreased G1 peak, a subdiploid peak indicative of cell death and a high percentage of cells in S/G2. By 96 h postinfection, the G1 population had declined substantially, indicating that cells previously in S/G2 had not cycled into G0. There also appeared to be a general decline in the DNA profiles consistent with the decline in viability (Fig. 1). In addition, a general observation was that cells appeared to traverse S phase and accumulate S/G2 DNA contents before cell death occurred. In contrast, ONYX-015-infected cells did not show any evidence of a subdiploid peak at either 48 or 96 h postinfection. Instead, by 48 h postinfection, cells had accumulated in late S/G2, and by 96 h postinfection, the majority of cells had acquired a greater than 4N DNA content. This increase in DNA content is not caused by doublet formation or cell clumping as all doublets/clumps were “gated out” of our DNA content analysis (see Fig. 3). This shift is also not caused by cell fusions because we would have observed integral shifts in DNA ploidy (i.e., distinct peaks at 6N, 8N, and so forth). Thus, the increased DNA content must be caused by a reinitiation of DNA replication without an intervening cell division. Consistent with this interpretation, addition of hydroxyurea (which inhibits initiation of DNA replication) prevented ONYX-015-infected cells from acquiring greater than 4N DNA contents (data not shown). This interpretation is also consistent with several reports demonstrating that adenovirus infection causes increases in cellular DNA content in rodent cells (19–21). One of these reports specifically excluded aggregates and cells with more than one nucleus using microspectrophotometry (19). Similar cell cycle profiles were observed in HepG2 cells after infection with ONYX-015 (data not shown).

These data provide further evidence for the existence of at least two distinct cell death phenotypes, one requiring E1b55k and one that does not. In addition, it appears that E1b55k functions in some way to relieve cell cycle abnormalities that lead to the accumulation of cells in late S/G2, and by so doing, it promotes cell death. However, the biochemical details of this cell death promotion are not yet clear and are the subject of ongoing investigation.

Formation of the E1b55k/p53 Complex Is Required for Cell Death. E1b55k is known to form a stable protein complex with p53 in both infected and adenovirus transformed cells (22–24). Because death occurred most rapidly under conditions in which both p53 and
E1b55k were present, we hypothesized that the E1b55k/p53 complex may be important for enhancing the cell death pathway, despite reports that E1b55k inhibits the action of p53 (25–28). An experiment was therefore carried out in which A549 cells were infected with a number of adenovirus insertion mutants (17) that express full-length E1b55k proteins, some of which are defective in p53 binding. Mutants H354, R443, and F484 retain an ability to bind p53, whereas mutants H180, A262, H326, and R309 are no longer able to bind (17). Results (Fig. 4) showed that mutants that retained the ability to complex with p53 were able to induce cell death with kinetics similar to those of wtAd5. By contrast, mutants defective for p53 binding induced cell death with delayed kinetics, similar to those observed for ONYX-015 and for p53 mutant cells.

Flow cytometric analysis of those mutants that failed to bind p53 (Fig. 5), showed similar profiles to those of the E1b55k-deficient virus ONYX-015 (Fig. 3) with DNA contents mostly greater than 4N. These data support the conclusion that in the absence of binding, cell death is delayed and DNA replication continues without an intervening cell division.

**Cell Death Occurs in a Cell Cycle-dependent Manner.** The flow cytometry data in Fig. 3 indicate that wtAd5-infected cells traverse S phase and acquire S/G2 DNA contents prior to cell death. More detailed kinetic data using flow cytometry confirm this interpretation (data not shown). In contrast, ONYX-015-infected cells accumulate S/G2 DNA contents but avoid death. These data suggest that adenovirus-induced p53-dependent death might be cell cycle dependent.
Thus, cell death as a consequence of wtAd5 infection may be explained by cells traversing S phase and becoming susceptible to death as they reach a critical point in the cell cycle. If this is so, then synchronizing infected cells such that a greater proportion traverse S phase as a cohort should generate a synchronous wave of cell death. To test this possibility, cells were synchronized by blocking at the G1/S boundary with hydroxyurea and, upon release, infecting them with wtAd5. Cell counts were performed over time periods sufficient to encompass two cell doublings after release from the block. These counts (Fig. 6) indicate that the first cell doubling in both infected and

<table>
<thead>
<tr>
<th>gated population</th>
<th>48 Hours</th>
<th>96 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td><img src="image" alt="control" /></td>
<td><img src="image" alt="control" /></td>
</tr>
<tr>
<td>wtAd5</td>
<td><img src="image" alt="wtAd5" /></td>
<td><img src="image" alt="wtAd5" /></td>
</tr>
<tr>
<td>dl 1520</td>
<td><img src="image" alt="dl 1520" /></td>
<td><img src="image" alt="dl 1520" /></td>
</tr>
</tbody>
</table>

Fig. 3. Cell cycle profiles of wtAd5- and ONYX-015-infected cells. Infected and mock-infected cells as for Fig. 2 were harvested and fixed. Cells were stained with propidium iodide and subjected to cell cycle analysis by flow cytometry (see “Materials and Methods”). Cells were gated to exclude doublets and cell aggregates using FL2 area versus FL2 width. Dot plots of the gated populations at 48 h postinfection are shown, and identical gates were applied to the 96 h postinfection population. DNA content analysis of uninfected controls and wtAd5- and ONYX-015-infected cells are shown at 48 and 96 h postinfection. Dotted lines, reference points showing the G1 and G2 populations for comparison between graphs.
uninfected cells proceeds normally with no evidence of cell death, presumably because they have insufficient time to express early viral proteins. However, at the second cell doubling, whereas uninfected cells doubled in number as expected, approximately half the wtAd5-infected cell population died. Although it is clear that these cells are no longer in synchrony at the point at which they die, cell death begins to occur in the wtAd5-infected population at the precise time that cell division begins in the uninfected population. Importantly, these events occur at similar rates.

These data suggest that the signal to undergo cell death is cell cycle dependent and that it occurs close to the point at which control cells undergo division. Thus, cells arrested by infection with E1b55k-deficient viruses may be protected from cell death because they never reach the position in the cell cycle at which infected cells die. Alternatively, it may be that in the absence of E1b55k, cells are insensitive to the cell death signal.

**DISCUSSION**

The widely held view of the action of p53 in response to adenoviral infection is that p53 is stabilized (29, 30), leading to its overexpression. This in turn leads to increased transcriptional activation of cell cycle regulators such as p21(Waf1/Cip1), which ultimately lead to cell cycle arrest (31). In addition, this up-regulation of p53 is responsible for the induction of apoptosis (30), although the mechanism by which p53 initiates this event is less well understood. However, adenoviruses counter these cellular defenses by expressing the E1b55k protein, which binds p53 and purportedly overcomes these growth restrictions, allowing an efficient adenoviral infection to take place (30). Based upon this premise, a new approach to cancer therapy has arisen using an adenovirus deficient for expression of the E1b55k protein (ONYX-015). It has been claimed that this virus selectively replicates in and kills tumor cells that do not express p53 (2, 3).

However, recent reports showing that ONYX-015 can replicate in cells expressing a wt p53 (5, 6) and an apparent requirement for p53 in adenovirus-induced cell death (4) appear to be inconsistent with the model proposed for ONYX-015-induced death.

The data presented here demonstrate that at least two distinct cell death pathways exist after infection with adenoviruses. These are distinguished by the kinetics of cell death and by a requirement for both E1b55k and p53. One pathway results in rapid cell death and is dependent on the presence of a functional p53 protein. In the absence of p53, cell death is delayed considerably, with a minimum of a 2–3 day lag time compared with infection of wt p53 expressing cells. In the absence of E1b55k (e.g., infection with ONYX-015), cell death was delayed similar to that observed for p53-deficient cells infected with wtAd5. In the absence of p53, E1b55k-deficient adenoviruses were unable to induce cell death in the 10-day time period of the experiments we conducted. Such data do not support the selective

![Fig. 4. Cell viability after infection with p53 binding mutants. A549 cells were infected with adenovirus insertion mutants (see "Materials and Methods") that either lacked (lightface dotted lines) or retained (lightface solid lines) their ability to bind p53. WtAd5 (boldface solid line), ONYX-015 (boldface dotted line), and uninfected controls (M) are also shown. Cell viability was assessed over 10 days as for Fig. 1. Data points, means of triplicate counts of viable cells expressed as a percentage of total cells; SE is shown if greater than 5% of the mean.](image)

![Fig. 5. Cell cycle profiles after infection with p53 binding mutants. Cells were infected with R309, A262, and H180 viruses, which express a mutant E1b55k protein unable to bind p53. At 4 days postinfection, these cells and mock-infected controls were harvested and fixed. Cells were stained with propidium iodide and subjected to cell cycle analysis (see "Materials and Methods"). Dotted lines show the G1 and G2 populations and are reference points for comparison between graphs.](image)

![Fig. 6. Cell number after synchronization and wtAd5 infection. A549 cells were synchronized using hydroxyurea (see "Materials and Methods") and infected with 10 CPEUs/cell of wtAd5 (■) or mock infected (○). Viable cell number was assessed as for Fig. 1 and plotted against time (solid lines). Viable cells as a percentage of total cells is also shown (dotted lines). Data points, means of triplicate counts; SE is shown if greater than 5% of the mean.](image)
targeting of p53-deficient cells by ONYX-015 but rather suggest that p53 and E1b55k are required for efficient induction of cell death by adenoviruses.

This is further supported by the finding that cells infected with E1b55k-deficient viruses fail to proliferate after infection but rather demonstrate an apparent block in cell division (Fig. 2, b and c). This is not caused by an equilibrium being reached between dying and living cells because viabilities remain high (Fig. 1). FACS analysis of these populations demonstrated that ONYX-015-infected cells accumulate with 4N and greater than 4N DNA contents but fail to undergo cell death. The lack of a subdiploid peak, present in the wtAd5-infected cells and characteristic of cell death, suggests that ONYX-015 cannot induce rapid cell death. These data support the conclusion that both p53 and E1b55k are necessary for the induction of the rapid death pathway. In the absence of either of these proteins, cell death is delayed considerably. These data also suggest that both p53 and E1b55k play a positive role in promoting the cell death process associated with adenoviral infection.

Because E1b55k has been shown previously to bind p53, we next considered whether the formation of the complex was necessary for the induction of cell death. To do this, we used a panel of insertion mutants, some of which disrupt p53 binding and others of which still bind p53 (17). Those mutants that retained an ability to bind p53 demonstrated rapid cell death with kinetics similar to those of wtAd5 (Fig. 4). In contrast, those mutants in which p53 binding was disrupted demonstrated a delayed cell death with kinetics that were remarkably similar to those of ONYX-015 (Fig. 4).

FACS analysis also demonstrated different death profiles dependent on the ability of the E1b55k protein to bind p53. Viruses that expressed E1b55k proteins that retained their ability to bind p53 accumulated in late S/G2 prior to death. These profiles were similar to those demonstrated for wtAd5 (Fig. 3). Viruses that expressed an E1b55k protein that was unable to bind p53 had DNA contents of 4N or greater than 4N (Fig. 5). These profiles are similar to those shown for ONYX-015 (Fig. 3) and suggest that cells are continuing to replicate their DNA but are apparently unable to divide. These data demonstrate that formation of the p53/E1b55k complex is essential for the induction of rapid cell death by adenoviruses and are the first evidence demonstrating a role for the E1b55k/p53 complex in promoting cell death.

Previous reports have shown that the adenovirus E4ORF6 protein binds E1b55k (32) and that E4ORF6 and p53 binding domains substantially overlap (24, 33). Thus, disruption of the p53 binding domain of E1b55k may equally affect the binding of both p53 and E4ORF6. However, the H180 virus, which disrupts p53 binding but does not disrupt E4ORF6 binding (34), still demonstrates an ability to abrogate the rapid cell death process (Fig. 4). This would argue against E4ORF6 being the protein responsible for the induction of p53-dependent cell death.

Another protein that is associated with and binds E1b55k is the recently identified E1B-AP5 (34). This protein also overlaps the p53 binding domain of E1b55k. Binding of E1B-AP5 is disrupted in the F484 mutant (34); however, this mutant retains its ability to bind p53, and our data demonstrate that it also retains its ability to induce rapid cell death (Fig. 4). These data would argue against a role for E1B-AP5 in the induction of p53-dependent cell death.

The data presented here and previously (4) indicate that rapid cell death requires the presence of both p53 and E1b55k and that these proteins must be able to complex. Thus, the original claim (2) that ONYX-015 was selective for tumor cells containing a mutant p53 is not supported by these data. Rather, they would suggest that wt p53-containing cells should be preferentially killed. However, ONYX-015 continues to show an ability to kill tumor cells in clinical trials (1, 35) despite the absence of a functional E1b55k protein and, in many cases, the absence of wt p53.

The basis of this p53-independent cell death may be a reduced ability of normal cells to either be infected or grow virus and therefore to induce cell death. This interpretation is supported by both our data (Fig. 1) and those of others (6) showing that HF5 require a much higher dosage of virus to induce similar kill rates to cell lines derived from human tumors. Alternatively, delayed death may be attributable to the apparent quiescent state of normal cells compared with their tumorous counterparts. It has been suggested previously (5) that cells are more readily infected when they are in S phase. Therefore, if cells are cycling rapidly, a high percentage of cells will be infected (because of their higher representation in S phase), whereas cells that are quiescent will be infected rather poorly. A further possibility is that death is simply a consequence of a delayed or impaired viral replicative process. However, we would argue against this being a simple relationship because replication of both wtAd5 and ONYX-015 has been demonstrated in p53 defective cells and cells expressing a wt p53 protein (4, 6). Also, similar replicative abilities have been demonstrated for p53 binding mutants in A549 cells regardless of whether or not they bind p53 (36).

Thus, the data presented here offer further evidence that ONYX-015 does not selectively kill tumor cells that are deficient for p53. In fact, the data suggest that the antitumor effects induced by ONYX-015 are attributable to the apparent selectivity of adenovirus infection for tumor cells. We would suggest that there is nothing unique about ONYX-015 in the context of tumor therapy. In fact, a more efficient destruction of tumor cells is likely to occur if wtAd5 were used as the therapeutic agent, because it should be able to induce cell death more efficiently in cells containing either a wt or mutant p53. The more efficient and faster death induced by wtAd5 should allow lower doses for equivalent results and therefore decrease the likelihood of possible side effects.

ACKNOWLEDGMENTS

We thank Dr. T. Shenk (Princeton) for dl 338 and Dr. A. Berk (University of California at Los Angeles) for dl 1520 (ONYX-015) and insertion mutants F484, R443, H354, R309, H180, A262, and H326.

REFERENCES


Efficient Induction of Cell Death by Adenoviruses Requires Binding of E1B55k and p53


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/60/10/2666

Cited articles
This article cites 34 articles, 21 of which you can access for free at:
http://cancerres.aacrjournals.org/content/60/10/2666.full#ref-list-1

Citing articles
This article has been cited by 20 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/60/10/2666.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/60/10/2666.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.