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Adenoviral-E2F-1 Radiosensitizes p53^{wild-type} and p53^{null} Human Prostate Cancer Cells

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Abstract

Purpose—E2F-1 is a transcription factor that enhances the radiosensitivity of various cell lines by inducing apoptosis. However, there are conflicting data concerning whether this enhancement is mediated via p53 dependent pathways. Additionally, the role of E2F-1 in the response of human prostate cancer to radiation has not been well characterized. In this study, we investigated the effect of Adenoviral-E2F-1 (Ad-E2F-1) on the radiosensitivity of p53^{wild-type} (LNCaP) and p53^{null} (PC3) prostate cancer cell lines.

Methods and Materials—LNCaP and PC3 cells were transduced with Ad-E2F-1, Adenoviral-Luciferase (Ad-Luc) control vector, or Adenoviral-p53 (Ad-p53). Expression of E2F-1 and p53 was examined by Western blot analysis. Annexin V and caspase 3 + 7 assays were performed to estimate the levels of apoptosis. Clonogenic survival assays were used to determine overall cell death. Statistical significance was determined by analysis of variance, using the Bonferroni method to correct for multiple comparisons.

Results—Western blot analysis confirmed the efficacy of transductions with Ad-E2F-1 and Ad-p53. Ad-E2F-1 transduction significantly enhanced apoptosis and decreased clonogenic survival in both cell lines. These effects were compounded by the addition of RT. Although E2F-1-mediated radiosensitization was independent of p53 status, this effect was more pronounced in p53^{wild-type} LNCaP cells. When PC3 cells were treated with Ad-p53 in combination with RT and Ad-E2F-1, there was at least an additive reduction in clonogenic survival. **Conclusions:** Our results suggest that Ad-E2F-1 significantly enhances the response of p53^{wild-type} and p53^{null} prostate cancer cells to radiation therapy, although radiosensitization is more pronounced in the presence of p53. Ad-E2F-1 may be a useful adjunct to radiation therapy in the treatment of prostate cancer.

Keywords

Adenoviral gene therapy; E2F-1; Prostate cancer; Radiation; Apoptosis

Introduction

E2F-1 is a transcription factor with multiple functions. Depending on the cellular milieu and predominant signal, it can act as either an oncogene or tumor suppressor (1, 2).

Overexpression of E2F-1 in the presence of Ras mutations has led to malignant transformation (3). Singh and colleagues demonstrated the ability of E2F-1 to transform rat embryo fibroblasts (4). However, other studies have suggested an opposing role of E2F-1. In animal models, Field *et al.* (5) and Yamasaki *et al.* (6) observed that E2F-1 knockout mice have an increased propensity to form tumors. Through interactions with various cell cycle regulators, it can act as a tumor suppressor by mediating cell cycle arrest, DNA repair, or apoptosis (7, 8).

Gene transfection experiments have demonstrated the ability of E2F-1 overexpression to induce tumor regression (9). Additionally, E2F-1 overexpression has been shown to enhance cellular radiosensitivity and increase cell death via apoptosis in certain cell lines (10–12). Even in cells with intact native E2F-1, exogenous overexpression of E2F-1 can also lead to cell-cycle arrest or apoptosis (13–15). Although it is clear that E2F-1 plays a central role in cell-cycle regulation and DNA repair, its function in prostate cancer is less certain (16). Moreover, the potential of E2F-1 administered via a gene therapy vector in conjunction with radiation has never been examined.

p53 is a much-studied tumor suppressor gene with some mechanisms of action analogous to E2F-1. It has been described as “guardian of the genome,” regulating cell-cycle progression, promoting repair of sublethal DNA damage, and inducing cell death when alterations are irreparable (17–19). Tumors with p53 mutations have been observed to be more aggressive and resistant to many therapeutic modalities, including radiation (20–25). As with E2F-1 gene transfer strategies, introduction of p53 into p53^{wild-type}, p53^{null}, or p53 mutant cell lines also enhances radiation response (26–32).

In this study, we investigated the effects of Adenoviral-E2F-1 (Ad-E2F-1) and Ad-p53 gene therapy on the responses of prostate cancer cells to radiation. Specifically, we asked the question: Does Ad-E2F-1 sensitize prostate cancer cells to radiation, and, if so, to what extent is this effect dependent on p53? The effect of Ad-E2F-1 on cell killing from radiation was examined in the p53^{wild-type} LN-CaP and p53^{null} PC3 human prostate cancer cell lines. Transduction experiments with both Ad-p53 and Ad-E2F-1 were performed to determine the effect of p53 replacement on the radiation response of PC3 cells to E2F-1 gene therapy.

Methods and Materials

Cell culture

LN-CaP and PC-3 cells from American Type Culture Collection (Rockville, MD) were maintained in Dulbecco's modified Eagle F12 medium supplemented with 10% fetal bovine

serum, 100 U/mL penicillin, 100 ug/mL streptomycin, and 4 mM glutamine. Cells were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO₂.

Transduction and protein expression analyses

Approximately 5×10^5 cells were plated on 10-cm dishes in duplicate for approximately 48 h. Adenovirus-5 (CMV promoter) constructs incorporating the E2F-1 (Ad-E2F-1) (33), p53 (Ad-p53) (31), and Luciferase (Ad-Luc) (32) genes were used to transduce cells at a multiplicity of infection (MOI) of 10, 25, or 50. Twenty-four hours after gene transduction, one set was irradiated with 6 Gy and reincubated for approximately 3 h while the duplicate set received no radiation therapy (RT). Cells were then harvested and lysed using buffer (50 mM Tris pH 7, 2% sodium dodecyl sulfate) containing proteinase inhibitors.

Western blot analyses were performed to confirm the success of transduction. Approximately 50–70 ug of protein from each cell lysate was electrophoresed on a 12% sodium dodecyl sulfate polyacrylamide gel. After transfer onto a polyvinylidenedifluoride membrane in a transblot apparatus and blocking with 5% low-fat dried milk, the blots were incubated overnight at 4°C with mouse monoclonal antibodies to E2F-1 (Oncogene, La Jolla, CA), pRb (Oncogene), p53 (Santa Cruz Biotech, Santa Cruz, CA), or p21 (Oncogene) at 0.1% antibody concentration in milk-blocking buffer. The membranes were washed and labeled with an anti-mouse horseradish peroxidase conjugated secondary antibody (Amersham, Buckinghamshire, UK) at room temperature for approximately 1 h. Detection by chemiluminescence was performed following the standard protocol in ECL user's guide (Amersham).

Measurement of apoptosis

Annexin V and Caspase-3 + 7 assays were performed to determine whether E2F-1 mediates cell killing via apoptosis. For each assay, 5×10^5 cells were transduced with Ad-E2F-1. Eighteen hours after gene transduction, one set was irradiated and reincubated while the other received no irradiation. After an additional 48 h, cells were harvested for Annexin V or caspase 3 + 7 assay. For the Annexin V assay (Guava Technologies Inc., Burlingame, CA), cells were labeled with Annexin V-Phycoerythrin (Annexin V-PE) and 7-amino-actinomycin D (7AAD) according to the manufacturer's instructions, and analyzed by flow cytometry on a GuavaPC personal flow cytometer. The cells that stained for Annexin V-PE and did not stain with 7AAD were considered to be in early apoptosis and the percentages of these cells are displayed in the tables. Caspase-3 + 7 activity was measured using a fluorometric substrate, Z-DEVD-Rhodamine (The Apo-ONE Homogeneous Caspase-3 + 7 Assay kit; Promega, Madison, WI). Harvested cells were mixed with 100 μ L of Homogenous Caspase-3 + 7 reagent in 96-well plates and incubated at room temperature for 18 h. Substrate cleavage was quantified fluorometrically at 485-nm excitation and 538-nm emission. Fluorescence was measured on a fluorescent plate reader (LabSystem Inc., Franklin, MA).

Clonogenic survival

The techniques for clonogenic survival assays have been described previously (34). For clonogenic survival assays, four sets of approximately 5×10^5 cells were plated onto sterile

10-cm dishes. Typically, after 48 h, 2×10^6 cells in each dish were available for gene transduction. The Ad-E2F-1, Ad-p53, and Ad-Luc vectors were maintained and diluted in phosphate-buffered saline until transduction. The cells in each dish were washed in phosphate-buffered saline to remove any residual serum that might bind viral particles and impede transduction. Appropriate dilutions of Ad-E2F-1 or Ad-p53 vector in 1 mL of solution were gently placed onto the monolayer of cells in each dish and incubated for 1 h. Control dishes with medium alone or with Ad-Luc were exposed to identical conditions. After incubation, 4 mL of control medium with serum was added to each dish and incubated overnight. At 24 h after viral exposure, three sets of dishes at each RT dose level were irradiated with a high dose rate cesium unit (^{137}Cs irradiator, Model 81-14R, JL, Shepherd & Associates, San Fernando, CA) to a total of 2, 4, or 6 Gy. Immediately after irradiation, cells were trypsinized, serially diluted, replated into 100-mm dishes, and incubated. After 14 days, colonies were stained with methylene blue and counted. Cell survival was adjusted for plating efficiency. For each radiation dose and viral titer, five experiments were performed, and the results were averaged.

Statistical analysis

Statistical significance between groups was assessed using analysis of variance, correcting for multiple comparisons using the Bonferroni method. Differences were considered statistically significant at the $p < 0.05$ level.

Results

The success of E2F-1 transduction by Ad-E2F-1 in LN-CaP cells was validated by Western blots. Figure 1 shows the expression of E2F-1, pRb, p53, and p21 in LNCaP cells treated with Ad-E2F-1 (25 MOI) or Ad-Luc (25 MOI), with or without 6 Gy single-dose RT. E2F-1 expression was evident in LNCaP cells transduced with Ad-E2F-1. The addition of RT did not appear to significantly increase E2F-1 expression. Although E2F-1 expression was not evident in cells treated with Ad-Luc or nonvector containing control medium (CM), this is the result of the chemiluminescence exposure conditions used in this study. Other experiments using the same cell line, but with different chemiluminescence exposure conditions, showed E2F-1 expression when these cells were incubated in CM or with Ad-Luc (data not shown). Overexpression of E2F-1 resulted in slight increases in p53 and p21 expression over that of the CM and the Ad-Luc controls. This effect of Ad-E2F-1 was not seen on pRb expression, although pRb expression was reduced by Ad-Luc exposure. The addition of RT enhanced levels of p53 and p21 in cells incubated in CM or with Ad-Luc. In the presence of E2F-1 overexpression, p53 and p21 expression were not further enhanced by RT.

Western blots of E2F-1, pRb, p53, and p21 expression in PC3 cells are shown in Fig. 2. PC3 cells were transduced with Ad-E2F-1 (50 MOI), Ad-p53 (50 MOI), or both (50 MOI each), with or without 6 Gy RT. Incubation in CM or transduction with Ad-Luc (50 MOI) served as controls. In PC3 cells, E2F-1 expression was detectable in Ad-E2F-1 transduced cells but not in the others under the chemiluminescence exposure conditions used here. Other experiments using the same cell line, but with different chemiluminescence exposure

conditions, showed E2F-1 expression when these cells were incubated in CM or with Ad-Luc (data not shown). Neither Ad-p53 nor RT significantly altered E2F-1 levels. pRb protein level was not affected by RT, but was slightly reduced by Ad-Luc, slightly increased by Ad-E2F-1, reduced by Ad-p53, and most obviously increased by Ad-E2F-1 plus Ad-p53. pRb expression was not affected by RT. As expected, p53 was not expressed in the p53^{null} PC3 cell line. Transduction with Ad-p53 resulted in p53 expression that was independent of RT or Ad-E2F-1 exposure. The expression of p21 paralleled that of p53.

Levels of apoptosis in LNCaP and PC3 cells were determined using Annexin V and caspase 3 + 7 assays (Table 1). The results of analysis of variance using the Bonferroni test to correct for multiple comparisons are shown. In LNCaP and PC3 cells, Ad-E2F-1 transduction did not significantly enhance Annexin V levels. When combined with RT, Ad-E2F-1 exposure resulted in a significant increase (at least additive) in Annexin V staining in LNCaP cells, but not PC3 cells. In contrast, Ad-E2F-1 alone was sufficient to significantly increase caspase 3 + 7 activity in LNCaP cells. The addition of RT to Ad-E2F-1 transduced LNCaP cells did not significantly enhance caspase 3 + 7 levels compared with Ad-E2F-1 alone. In the p53^{null} PC3 cell line, Ad-E2F-1 alone did not significantly enhance caspase 3 + 7 activity over that of Ad-Luc, but did result in significantly increased activity when combined with RT.

Clonogenic survival plating efficiencies of LNCaP and PC3 cells are listed in Table 2. Each experiment was repeated five times, and the mean and standard deviations were calculated for each set of experiments. To adjust for variations in experimental conditions, the plating efficiencies of cells transduced with Ad-Luc, Ad-E2F-1, Ad-p53, and the combination of Ad-E2F-1 + Ad-p53 were each normalized to their respective controls and expressed as ratios of the mean. For LNCaP and PC3 cells incubated in control medium, the mean ($\pm$ SD) plating efficiencies were 15.5% ($\pm$ 5.6%) and 37.7% ($\pm$ 17.7%), respectively. In general, increasing the MOI from 10 to 50 reduced the plating efficiency for all of the viral vectors used. One exception was for PC3 cells treated with Ad-p53 alone, in which there was no statistically significant difference in the plating efficiencies at 10, 25, or 50 MOI. Ad-p53 alone or in combination with Ad-E2F-1 was effective in reducing PC3 cell plating efficiency relative to Ad-Luc.

In LNCaP cells, the addition of Ad-E2F-1 did not significantly affect plating efficiency relative to Ad-Luc. However, Ad-E2F-1 significantly reduced PC3 cell survival compared with Ad-Luc at MOIs of 25 and 50.

The effects of E2F-1 on cell survival normalized to plating efficiency in the absence of RT are shown in Fig. 3 and Fig. 4. Ad-E2F-1 significantly radiosensitized LNCaP and PC3 cells. In LNCaP cells, radiosensitization was observed at all RT dose levels when Ad-E2F-1 at MOI of 10 was added (Table 3). In the PC3 cell line, significant radiosensitization by Ad-E2F-1 required an MOI of 50; neither an Ad-E2F-1 MOI of 10 nor 25 was sufficient to produce significant differences compared with controls (Table 4). Exposure of p53^{null} PC3 cells to Ad-p53 at an MOI of at least 25 also significantly increased the radiation response of PC3 cells (Table 5). When p53 replacement with Ad-p53 was combined with Ad-E2F-1

there was at least an additive effect on PC3 cell radiosensitization that was most evident at an MOI of 25 for each vector (Table 6, Fig. 4).

Discussion

Prostate cancer remains a leading killer of men. Although widespread use of prostate-specific antigen and digital rectal exam screenings have led to earlier diagnosis, a significant number of men still present with high-risk clinically localized disease (35, 36). About half of such men will experience recurrence after definitive therapy. Local recurrence remains a major cause of failure despite improvements in radiation treatment delivery, radiation dose escalation, and combined treatment with androgen deprivation (37-39). Targeted biologic therapy holds promise for improving radiation response.

In this study, we demonstrated the efficacy of Ad-E2F-1 in enhancing the radiosensitivity of two prostate cancer cell lines using apoptotic and clonogenic survival assays. Annexin V and caspase 3 + 7 assays, used to estimate cell death, showed that Ad-E2F-1 transduction increased apoptosis. Although Ad-E2F-1 enhanced Annexin V and caspase 3+7 activities in both cell lines, these effects were more significant in LNCaP cells. Using clonogenic survival assays, we demonstrated that Ad-E2F-1 transduction substantially increased overall cell death in p53^{wild-type} LNCaP cells and p53^{null} PC3 cells. Even though p53 was not required for radiosensitization from E2F-1 overexpression, higher titers of Ad-E2F-1 were required to produce similar decrements in cell survival in PC3 cells. This difference in radiosensitization from Ad-E2F-1 between LNCaP and PC3 cells may be due in part to other downstream factors and not necessarily on codependence of E2F-1 and p53. Nevertheless, when PC3 cells were cotransduced with Ad-E2F-1 and Ad-p53, there was at least an additive increase in radiation induced overall cell death.

Our results in prostate cancer cell lines are in keeping with other investigations involving different cell lines. In serum-starved fibroblasts, Qin and colleagues (40) demonstrated that E2F-1 overexpression led to p53-dependent apoptosis. Bargou *et al.* (41) showed that inhibition of E2F-1 in a normal breast epithelial cell line inhibited apoptosis and induced tumor growth in SCID mice. Similarly, Shan and Lee (9) confirmed that REF52 and RAT2 cell lines lost their ability to undergo apoptosis when E2F-1 was mutated. A potential mechanism forwarded by Kowalik and colleagues (42) was that E2F-1 promotes p53 dependent apoptosis by sequestering MDM2 to prevent ubiquitination and degradation of p53. As in our study, they showed that overexpression of E2F-1 led to accumulation of p53. Studies by Hsieh *et al.* (43) and Kowalik *et al.* (42) independently confirmed that overexpression of MDM2 limited native E2F-1's ability to induce p53-dependent apoptosis.

p53 may play a key role in E2F-1-mediated apoptosis; however, other studies have suggested a p53-independent mechanism similar to our results with p53^{null} PC3 cell line. Pruschy and colleagues (10) demonstrated that over-expression of E2F-1 increased radiation sensitivity in a p53-negative fibrosarcoma cell line. Macleod *et al.* (44) showed that unregulated E2F-1 activity in a mouse peripheral nervous system lacking functional pRb led to increased apoptosis independent of p53 status. A testicular tumor model studied by

Holmberg *et al.* (45) also suggested that the apoptotic cascade was intact regardless of p53 status.

In conclusion, we have shown for the first time in human prostate cancer cell lines that Ad-E2F-1 is a potent radiosensitizer, particularly when wild-type p53 is present. Because most early prostate cancers express functional p53, Ad-E2F-1 should have considerable activity. p53 mutations are much more prevalent in locally advanced cancers and our results suggest at least an additive radiosensitizing effect of Ad-p53 and Ad-E2F-1 in prostate cancer cells lacking functional p53. In this setting, p53 replacement with Ad-p53 might be a useful adjunct to Ad-E2F-1 and RT. These findings suggest a potential role of Ad-E2F-1 gene therapy in the radiotherapeutic management of prostate cancer.

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Fig. 1. LNCaP Western blots. LNCaP cells were transduced with 25 multiplicity of infection of Ad-E2F-1 or Ad-Luc. After 24 h of gene transduction, cells were irradiated with 6 Gy and lysed 3 h later. Abbreviations: CM = control medium; RT = radiation therapy; Ad-E2F-1 = Adenoviral-E2F-1; Ad-Luc = Adenoviral Luciferase.

Fig. 2.
PC3 Western blots. PC3 cells were transduced with 50 multiplicity of infection of Ad-E2F-1, Ad-p53, or both. Ad-Luc and control medium alone served as controls. After 24 h of gene transduction, cells were irradiated with 6 Gy and lysed 3 hours later. Abbreviations: CM = control medium; RT = radiation therapy; Ad-Luc = Adenoviral-Luciferase; Ad-E2F-1 = Adeno-viral-E2F-1; Ad-p53 = Adenoviral-p53.

Fig. 3. Effect of Adenoviral-E2F-1 (Ad-E2F-1) on clonogenic survival of p53^{wild-type} (LNCaP) and PC3 cells. LNCaP and PC3 cells were transduced with Ad-E2F-1 at multiplicity of infection of 10, 25, and 50.

Fig. 4. Effect of Ad-p53 and 1 Ad-E2F-1 + Ad-p53 on clonogenic survival of PC3 cells. PC3 cells were transduced with Ad-p53 alone or in combination with Ad-E2F-1 at multiplicity of infection (MOI) of 10, 25, and 50. The Adenoviral-Luciferase control was administered at MOIs of 10, 25, and 50.

Table 1

Apoptosis assays in LNCaP and PC3 prostate cancer cells

Groups	LNCaP				PC3			
	Annexin V*	Bonferroni test [†]	Caspase 3 + 7*	Bonferroni test [†]	Annexin V*	Bonferroni test [†]	Caspase 3 + 7*	Bonferroni test [†]
Control	5.4 ± 0.8	6	204 ± 68	1, 6	5.2 ± 1.9	6	65 ± 25	6
AdLuc	6.8 ± 2.9	7	446 ± 225	2, 7	5.7 ± 2.2	7	128 ± 57	7
Ad-E2F-1	10.4 ± 2.5	5	1507 ± 472	1, 2, 3, 4	4.1 ± 1.5	3, 5	723 ± 408	5
Control + RT	8.0 ± 1.4	8	440 ± 253	3, 8	8.2 ± 2.6	3	137 ± 70	8
AdLuc + RT	9.9 ± 1.2	9	499 ± 317	4, 9	8.1 ± 1.6	NS	194 ± 119	9
Ad-E2F-1 + RT	18.1 ± 3.1	5, 6, 7, 8, 9	2128 ± 77	6, 7, 8, 9	10.9 ± 1.8	5, 6, 7	1516 ± 782	5, 6, 7, 8, 9

Abbreviations: RT = radiotherapy; Ad-Luc = Adenoviral-Luciferase; Ad-E2F-1 = Adenoviral-E2F-1.

* Annexin V (percent of Annexin V-PE positive and 7AAD negative) and Caspase 3 + 7 (relative fluorescence units) apoptosis assay values are tabulated as mean of five experiments ± standard deviation.

[†] Bonferroni test: 1, $p < 0.05$ for Ad-E2F-1 vs. control; 2, $p < 0.05$ for Ad-E2F-1 vs. Ad-Luc; 3, $p < 0.05$ for Ad-E2F-1 vs. control + RT; 4, $p < 0.05$ for Ad-E2F-1 vs. Ad-Luc + RT; 5, $p < 0.05$ for Ad-E2F-1 vs. Ad-E2F-1 + RT; 6, $p < 0.05$ for Ad-E2F-1 + RT vs. control; 7, $p < 0.05$ for Ad-E2F-1 + RT vs. Ad-Luc; 8, $p < 0.05$ for Ad-E2F-1 + RT vs. control + RT; 9, $p < 0.05$ for Ad-E2F-1 + RT vs. Ad-Luc + RT.

Table 2
Effect of Ad-E2F-1, Ad-p53, and Ad-Luc on LNCaP and PC3 plating efficiencies using analysis of variance

LNCaP	Ad-Luc/C*	Ad-E2F-1/C*	T-test [†]
10 MOI	0.68 ± 0.20	0.50 ± 0.06	0.200
25 MOI	0.33 ± 0.08	0.23 ± 0.07	0.159
50 MOI	0.29 ± 0.23	0.12 ± 0.04	0.166
PC3	Ad-Luc/C*	Ad-E2F-1/C*	Ad-p53/C* (Ad-E2F1 + Ad-p53)/C* Bonferroni [‡]
10 MOI	0.85 ± 0.20	0.79 ± 0.36	0.11 ± 0.00 0.13 ± 0.14 2, 3, 4, 5
25 MOI	0.79 ± 0.14	0.48 ± 0.26	0.06 ± 0.04 0.09 ± 0.16 1, 2, 3, 4, 5
50 MOI	0.79 ± 0.17	0.14 ± 0.12	0.32 ± 0.23 0.00 ± 0.00 1, 2, 4

Abbreviations: Ad-Luc = Adenoviral-Luciferase; Ad-E2F-1 = Adenoviral-E2F-1; C = Control; MOI = multiplicity of infection.

* Plating efficiency for control was tabulated as mean percent survival of five experiments ± standard deviations (SD). The plating efficiency ratios were tabulated as ratios of the means ± SD of Ad-Luc or Ad-E2F-1 over means of the corresponding controls.

[†] Bonferroni test: 1, $p < 0.05$ for Ad-E2F-1/C vs. Ad-Luc/C; 2, $p < 0.05$ for Ad-p53/C vs. Ad-Luc/C; 3, $p < 0.05$ for Ad-p53/C vs. Ad-E2F-1/C; 4, $p < 0.05$ for (Ad-E2F-1 + Ad-p53)/C vs. Ad-Luc/C; 5, $p < 0.05$ for (Ad-E2F-1 + Ad-p53)/C vs. Ad-E2F-1/C; 6, $p < 0.05$ for (Ad-E2F-1 + Ad-p53)/C vs. Ad-p53/C.

Table 3
Comparison of clonogenic percent survival of Ad-E2F-1 transduced LNCaP cells using analysis of variance

10 MOI				
RT dose	Control[*]	Ad-Luc[*]	Ad-E2F-1[*]	Bonferroni test[†]
2 Gy	27.16 ± 1.02	24.79 ± 1.59	17.20 ± 0.72	1, 2
4 Gy	6.86 ± 0.34	6.43 ± 0.65	3.15 ± 0.22	1, 2
6 Gy	1.43 ± 0.10	1.23 ± 0.24	0.37 ± 0.05	1, 2
25 MOI				
RT dose	Control[*]	Ad-Luc[*]	Ad-E2F-1[*]	Bonferroni test[†]
2 Gy	31.76 ± 8.28	32.53 ± 9.10	16.84 ± 3.39	NS
4 Gy	7.04 ± 2.87	5.24 ± 2.48	1.97 ± 0.95	NS
6 Gy	1.01 ± 0.47	0.90 ± 0.17	0.17 ± 0.15	1
50 MOI				
RT dose	Control[*]	Ad-Luc[*]	Ad-E2F-1[*]	Bonferroni test[†]
2 Gy	43.00 ± 5.17	41.48 ± 5.68	9.26 ± 4.63	1, 2
4 Gy	11.20 ± 3.63	8.05 ± 3.55	0.85 ± 0.38	1, 2
6 Gy	1.46 ± 0.89	1.43 ± 1.25	0.02 ± 0.02	NS

Abbreviations: Ad-Luc = Adenoviral-Luciferase; Ad-E2F-1 = Adenoviral-E2F-1; MOI = multiplicity of infection; NS = Nonsignificant; R = radiation therapy.

^{*} Clonogenic percent survival is tabulated as mean of 5 experiments ± standard deviations.

[†] Bonferroni test: 1, $p < 0.05$ for Ad-E2F-1 vs. control; 2, $p < 0.05$ for Ad-E2F-1 vs. Ad-Luc; NS, $p > 0.05$.

Table 4
Comparison of clonogenic percent survival of Ad-E2F-1 transduced PC3 cells using analysis of variance

10 MOI				
RT dose	Control[*]	Ad-Luc[*]	Ad-E2F-1[*]	Bonferroni test[†]
2 Gy	28.02 ± 1.76	26.10 ± 1.76	23.03 ± 6.66	NS
4 Gy	4.70 ± 2.60	4.50 ± 2.36	2.24 ± 1.22	NS
6 Gy	1.01 ± 1.12	0.87 ± 0.98	0.13 ± 0.14	NS
25 MOI				
RT dose	Control[*]	Ad-Luc[*]	Ad-E2F-1[*]	Bonferroni test[†]
2 Gy	31.76 ± 8.28	32.53 ± 9.10	16.84 ± 3.39	NS
4 Gy	10.31 ± 4.74	8.65 ± 3.81	5.04 ± 3.38	NS
6 Gy	1.79 ± 1.48	0.67 ± 0.90	0.11 ± 0.12	NS
50 MOI				
RT dose	Control[*]	Ad-Luc[*]	Ad-E2F-1[*]	Bonferroni test[†]
2 Gy	39.74 ± 2.61	38.43 ± 5.66	15.98 ± 5.20	1, 2
4 Gy	6.60 ± 2.67	4.82 ± 1.61	1.13 ± 0.84	1, 2
6 Gy	1.20 ± 0.49	0.80 ± 0.54	0.06 ± 0.07	1, 2

Abbreviations: Ad-Luc = Adenoviral-Luciferase; Ad-E2F-1 = Adenoviral-E2F-1; MOI = multiplicity of infection; NS = nonsignificant; RT = radiation therapy.

^{*} Clonogenic percent survival is tabulated as mean of five experiments ± standard deviations.

[†] Bonferroni test: 1, $p < 0.05$ for Ad-E2F-1 vs. control; 2, $p < 0.05$ for Ad-E2F-1 vs. Ad-Luc; NS, $p > 0.05$.

Table 5
Comparison of clonogenic percent survival of Ad-p53 transduced PC3 cells using analysis of variance

10 MOI				
RT dose	Control*	Ad-Luc*	Ad-p53*	Bonferroni test [†]
2 Gy	58.13 ± 2.34	60.30 ± 5.90	41.89 ± 4.24	NS
4 Gy	4.70 ± 2.60	4.50 ± 2.36	2.24 ± 1.22	NS
6 Gy	6.97 ± 0.69	6.89 ± 1.47	3.58 ± 0.00	NS
25 MOI				
RT dose	Control*	Ad-Luc*	Ad-p53*	Bonferroni test [†]
2 Gy	51.75 ± 5.25	46.98 ± 1.01	29.87 ± 3.34	1, 2
4 Gy	15.41 ± 1.79	14.60 ± 0.65	7.45 ± 1.09	1, 2
6 Gy	3.66 ± 1.33	2.44 ± 0.76	0.43 ± 0.35	1
50 MOI				
RT dose	Control*	Ad-Luc*	Ad-p53*	Bonferroni test [†]
2 Gy	46.85 ± 4.25	45.91 ± 1.27	15.73 ± 5.55	1, 2
4 Gy	15.48 ± 2.02	14.91 ± 1.56	1.33 ± 0.66	1, 2
6 Gy	2.96 ± 1.19	1.98 ± 0.81	0.11 ± 0.04	1, 2

Abbreviations: Ad-Luc = Adenoviral-Luciferase; Ad-p53 = Adenoviral-p53; MOI = multiplicity of infection; NS = nonsignificant; RT = radiation therapy.

* Clonogenic percent survival is tabulated as mean of five experiments ± standard deviation.

[†] Bonferroni test: 1, $p < 0.05$ for Ad-p53 vs. control; 2, $p < 0.05$ for Ad-p53 vs. Ad-Luc; NS, $p > 0.05$.

Table 6
Comparison of clonogenic percent survival of Ad-E2F-1 and Ad-p53 transduced PC3 cells using analysis of variance

10 MOI				
RT dose	Control*	Ad-Luc*	Ad-E2F-1 + Ad-p53*	Bonferroni test[†]
2 Gy	48.61 ± 10.28	65.45 ± 12.92	32.62 ± 26.13	NS
4 Gy	21.92 ± 8.90	14.64 ± 6.99	4.54 ± 2.73	1
6 Gy	5.20 ± 4.25	5.00 ± 4.15	0.63 ± 0.84	NS
25 MOI				
RT dose	Control*	Ad-Luc*	Ad-E2F-1 + Ad-p53*	Bonferroni test[†]
2 Gy	52.98 ± 1.88	46.85 ± 6.15	18.33 ± 6.33	1, 2
4 Gy	13.45 ± 3.53	13.43 ± 0.36	2.61 ± 2.13	1, 2
6 Gy	2.77 ± 0.87	2.05 ± 1.15	0.12 ± 0.16	NS
50 MOI				
RT dose	Control*	Ad-Luc*	Ad-E2F-1 + Ad-p53*	Bonferroni test[†]
2 Gy	47.99 ± 2.99	49.68 ± 5.26	13.26 ± 4.45	1, 2
4 Gy	14.31 ± 1.19	13.31 ± 0.41	1.28 ± 1.67	1, 2
6 Gy	N/A	N/A	N/A	N/A

Abbreviations: Ad-Luc = Adenoviral-Luciferase; Ad-E2F-1 = Adenoviral-E2F-1; Ad-p53 = Adenoviral-p53; MOI = multiplicity of infection; NS = nonsignificant; N/A = not available; RT = radiation therapy.

* Clonogenic percent survival is tabulated as mean of five experiments ± standard deviation.

[†] Bonferroni test: 1, $p < 0.05$ for Ad-E2F-1 + Ad-p53 vs. control; 2, $p < 0.05$ for Ad-E2F-1 + Ad-p53 vs. Ad-Luc; NS, $p > 0.05$.