Inhibition of Repair of Radiation-Induced DNA Damage Enhances Gene Expression from Replication-Defective Adenoviral Vectors

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Abstract
Radiation has been shown to up-regulate gene expression from adenoviral vectors in previous studies. In the current study, we show that radiation-induced dsDNA breaks and subsequent signaling through the mitogen-activated protein kinase (MAPK) pathway are responsible, at least in part, for this enhancement of transgene expression both in vitro and in vivo. Inhibitors of ataxia-telangiectasia–mutated, poly(ADP-ribose) polymerase–mutated, and DNA-dependent protein kinase (DNA-PK)–mediated DNA repair were shown to maintain dsDNA breaks (γH2AX foci) by fluorescence-activated cell sorting and microscopy. Inhibition of DNA repair was associated with increased green fluorescent protein (GFP) expression from a replication-defective adenoviral vector (Ad-CMV-GFP). Radiation-induced up-regulation of gene expression was abrogated by inhibitors of MAPK (PD980059 and U0126) and phosphatidylinositol 3-kinase (LY294002) but not by p38 MAPK inhibition. A reporter plasmid assay in which GFP was under the transcriptional control of artificial Egr-1 or cytomegalovirus promoters showed that the DNA repair inhibitors increased GFP expression only in the context of the Egr-1 promoter.

Introduction
Data emerging from initial phase I studies of viral vectors for cancer gene therapy have clearly shown that, in their present form, these agents are unlikely to be effective as single-agent stand-alone therapeutics. Consequently, there has been a shift in emphasis in preclinical and clinical studies toward using viral vectors as part of a combination treatment strategy with radiotherapy and/or chemotherapy (1). Appreciation of this fact has led researchers to engineer vectors (or ancillary therapeutics) with the capacity to maximize the effect of the standard treatment modality. For example, adenoviral vectors that express prodruk activating enzymes (e.g., thymidine kinase or cytosine deaminase) can be used in the context of radiation delivery to exploit the radiosensitizing effects of ganciclovir triphosphate and 5-fluorouracil, respectively (2–5). Alternatively, adenovirally mediated generation of radiosensitizing cytokines (e.g., tumor necrosis factor α) can be used to enhance the effects of radiation (6). These approaches are currently undergoing evaluation in clinical trials (7–12).

However, combining viral gene delivery with standard therapeutics also opens up the possibility of unanticipated interactions, some of which may be therapeutically useful. Several studies have reported increased expression of transgenes from adenoviral vectors following exposure to radiation and other DNA-damaging agents. Qian and colleagues (13) reported a 6.5- to 24.4-fold radiation-induced increase in adenoviral uptake and transgene expression in colon cancer cells in vitro and in vivo. Egami and colleagues (14) reported a 4.5-fold increase in NK4 (a hepatocyte growth factor antagonist) expression in pancreatic tumor cells. Seidman and colleagues (15) showed increased cell-associated adenovirus in cells synchronized in M phase by exposure to paclitaxel and nocodazole. Other studies have reported enhanced oncolysis from replication-competent adenovirus vectors following exposure to taxanes and vincristine, and these findings have been attributed to activation of cell signaling pathways that lead to cell cycle arrest and apoptosis (16). Increased gene expression from a cytomegalovirus (CMV) promoter has been reported in adenoviral construct following exposure to the DNA-damaging agents cisplatin (17, 18) and N-acetoxy-acetylaminofluorine (18) in a variety of tumor cell lines. Interestingly, this effect was most pronounced in xeroderma pigmentosum and Cockayne syndrome fibroblasts that are deficient in the transcription-coupled repair pathway of nucleotide excision repair. The authors hypothesized that the effect was mediated by persistent DNA damage (18).

The studies reported above were based on constitutive viral promoters. However, a separate, but related, strand of research in the use of adenoviral vectors and radiation has aimed to exploit elements derived from the Egr-1 promoter to control viral gene expression spatially and temporally. C(A/T)/G (C ARG) elements within the Egr-1 promoter are responsive to ionizing radiation and reactive oxygen species (19, 20). Datta and colleagues (19) showed abrogation of radiation- and peroxide-induced Egr-1–driven gene expression by the antioxidant N-acetyl-l-cysteine. The potential therapeutic utility of this system was shown in the context of a replication-defective adenoviral vector in which tumor necrosis...
factor-α was under the transcriptional control of an Egr-1–derived promoter (20). Enhanced radiation response in tumor, but not normal, tissue was shown in a nude mouse model.

Therefore, if adenovirus-mediated gene expression is influenced by DNA-damaging agents and by maintenance of DNA damage, there seems to be an opportunity to exploit this situation for therapeutic gain by combining radiation, adenoviral gene delivery, and drugs that inhibit repair of radiation-induced DNA damage. Such a strategy would have the potential to gain therapeutic benefit from complementary actions of the different components of the combination. For example, radiation may increase levels of adenoviral gene expression and this, in turn, may enhance the cytotoxicity of the radiation. At the same time, inhibition of repair of radiation-induced DNA damage may further enhance both gene expression and radiation-induced cytotoxicity.

In this article, we describe experiments designed to test the effect of inhibitors of DNA damage repair on gene expression from replication-deficient adenoviral vectors expressing green fluorescent protein (GFP) or luciferase (Luc). These data show that such an approach has the potential for development as a therapeutic strategy.

Materials and Methods

Cell lines. HCT116 (colorectal cancer), SIHN-5B, SIHN-011A, SIHN-011B, HN-5, and Cal-27 (all head and neck cancer) cells were cultured in DMEM containing 5% (v/v) FCS, 1% (v/v) glutamine, and 0.5% (v/v) penicillin/streptomycin. H1299 (lung cancer) cells were grown in the same medium under continuous selection with puromycin. Cell lines were maintained at 37°C and 5% CO2 in a humidified incubator.

Adenoviral vectors and infections. Stocks of replication-defective adenoviral vectors expressing GFP (Ad-CMV-GFP) or Luc (Ad-RSV-Luc) were stored at −80°C. Infections were performed at 70% to 75% confluence (−1 × 106 cells in 24-well plates/5 × 105 cells in six-well plates) in DMEM supplemented with 2% FCS. Cells were subsequently incubated at 37°C for at least 4 h followed by addition of fresh medium. Cells were subjected to functional analysis at fixed time points following infection.

Egr-1 reporter plasmid constructs. Plasmid constructs in which GFP was under the transcriptional control of either a CMV (pCMV-GFP) or a synthetic promoter (pE9-GFP) containing nine tandem repeats of a C(A/T)6GG motif (CARG element; refs. 21, 22) were amplified using Escherichia coli transformation and selection of antibiotic-resistant clones. Plasmid DNA was subsequently isolated and purified using a DNA MiniPrep kit (Qiagen Biosciences) and the plasmid concentration was determined by spectrophotometry at 260 and 280 nm. HCT116 cells were grown in six-well plates (2.5 × 103 per well) and transfected with 2 to 5 μg of either pCMV-GFP or pE9-GFP using Lipofectamine (Invitrogen Ltd.) for 90 min at room temperature to fluorescence-activated cell sorting (FACS) analysis for GFP expression.

DNA repair inhibitors. Poly(ADP-ribose) polymerase inhibitor (PARPi; Ku 0058948, C20H27N2O5F; ref. 23), ataxia-telangiectasia mutated inhibitor (ATMi; Ku 0055993, C21H17NO3S; ref. 24) and the DNA-dependent protein kinase inhibitor (DNA-PKi; Ku 0057788/NU7441, C25H19NO3S, and Ku 0060648, a water-soluble version of Ku 0057788/NU7441; ref. 25) were supplied by KuDOS Pharmaceuticals. PARPi stocks were stored at 1 mmol/L in 100% DMSO (VWR International Ltd.) and working concentrations of 1 to 10 μmol/L were used. ATMi and DNA-PKi stocks were stored at 1 mmol/L in 100% DMSO (VWR International Ltd.) and working concentrations of 1 to 5 μmol/L were used for ATMi and 1 μmol/L for DNA-PKi. All stock solutions were stored at −20°C and protected from light.

Signal transduction inhibitors. Mitogen-activated protein kinase kinase (MAPKK/MEK)extracellularkinase-regulatedkinase(ERK)inhibitorsPD980059 and U0126 were obtained from Tocris Biosciences and Calbiochem Biosciences, respectively. Phosphoinositide 3-kinase (PI3K) inhibitor, LY294002, and p38/stress-activated protein kinase (SAPK) inhibitor, SB202190, were obtained from Calbiochem Biosciences. All compounds were stored as 10 mmol/L stock solutions according to the manufacturers’ instructions.

Cell irradiation. Irradiations were performed using a Pantak H.F. 320 kV X-ray machine (AGO X-RAY Ltd.). Before irradiation of cells, the dose rate was determined using a Farmer Sub-Standard X-ray dosimeter MK.2/S3 according to the manufacturer’s instructions. The dose rate for irradiation was 6.6 to 6.8 Gy/min at 240 kVp and 10 mA. Cells were irradiated in 24-well plates (BD Labware) or 96-well plates (Nunc) or in 75 cm<sup>2</sup> tissue culture flasks (BD Biosciences) depending on the experimental design.

FACS for GFP expression. For Ad-CMV-GFP, 1 × 10<sup>5</sup> cells were infected in 75 cm<sup>2</sup> tissue culture flasks. For pCMV-GFP and pE9-GFP, 2.5 × 10<sup>5</sup> cells per well were transfected in six-well plates. At distinct time points following infection or transfection, the cell monolayer was trypsinized, washed vigorously thrice with 4 mL PBS, and resuspended as a single-cell suspension at 1 × 10<sup>5</sup> cells/ml. Propidium iodide was added to each sample at 1 μg/ml. Samples were analyzed on a FACSscan flow cytometer (Becton Dickinson). The percentage of cells exhibiting green fluorescence and the mean fluorescence of the sample, outside the area of background fluorescence defined in the untransduced control, were used as estimates of GFP expression. Data were normalized to the unirradiated sample to provide an estimate of fold increase in the level of GFP expression.

γH2AX focus assay. Phospho-γH2AX foci were used to quantify radiation-induced double-strand breaks (DSB). For FACS-based assay, 3 × 10<sup>5</sup> cells were seeded in 75 cm<sup>2</sup> tissue culture flasks, and 12 to 16 h later, the cells were treated with 1 μmol/L ATMi, 1 μmol/L PARPi, 1 μmol/L ATM, or DNA-PKi. Irradiation (5 Gy) was delivered 1 h after the application of DNA repair inhibitors. At 24 h, cells were trypsinized and fixed with 70% ice-cold ethanol, resuspended in a single-cell suspension, and incubated for 60 min at room temperature with 1:500 dilution of primary mouse anti-γH2AX antibody (Upstate Biotechnology) diluted in PBS with 4% FCS and 0.1% Triton X-100. Cells were washed thrice and incubated for 30 min at room temperature with FITC-conjugated anti-mouse secondary antibody (1:500). Cells were washed thrice and resuspended as a single-cell suspension of 1 × 10<sup>5</sup>/ml. Twenty thousand events were collected and analyzed on a FACScalibur flow cytometer. Data were normalized to unirradiated control to provide an estimate of fold increase in the level of DSB with radiation and other agents.

For confocal microscopic evaluation of γH2AX focus formation, cells were grown on coverslips in six-well plates, treated with DNA repair inhibitors, and irradiated 1 h later. They were subsequently returned to the incubator at 37°C for 24 h before being fixed in 4% paraformaldehyde in PBS for 1 h at room temperature. Following fixation, cells were kept in 1% (v/v) bovine serum albumin and 2% FCS in PBS at 4°C until stained. At the time of staining, cells at room temperature were permeabilized with a covering volume of 0.2% (v/v) Triton X-100 in PBS for 10 min, washed, and incubated with IFF in PBS for 10 min. Coverslips were removed and inverted onto 50 μL of mouse anti-phosphohistone γH2AX antibody diluted 1:2,000 in IFF for 1 h at room temperature. Coverslips were washed thrice and then inverted onto 50 μL of FITC-conjugated donkey anti-mouse secondary antibody diluted 1:250 in IFF for 40 min. Cells were washed thrice with TO-PRO-3 iodide (Molecular Probes) diluted 1:10,000 in PBS in a six-well plate on a rocking platform. Coverslips were inverted onto a drop of DakoCytomation fluorescent mounting medium on glass slides and viewed with a Nikon Eclipse E600 confocal microscope.

In vivo assessment of the effect of irradiation and DNA repair inhibition on Luc expression. Tumor xenografts were established by injecting 1 × 10<sup>6</sup> HCT116 cells into the right flank of 6-wk-old female MF1 nude mice. At 2 wk, animals with well-formed (5–8 mm diameter) tumors were anesthetized by i.p. injection of 1:1:4 mixture of Hypnorm (0.315 mg/ml fentanyl citrate, 10 mg/ml fluanisone; Janssen-Cilag Ltd.), Hypnovel (5 mg/ml midazolam; Roche Products Ltd.), and water for injection BP (Fresenius Health Care Group). Anaesthetized animals were positioned in an irradiation jig with the s.c. tumors exposed under an X-ray machine (AGO X-RAY Ltd.). Before irradiation of tumors, the dose rate was determined using a Farmer Sub-Standard X-ray dosimeter MK.2/S3 according to the manufacturer’s instructions. The dose rate for irradiation was 6.6 to 6.8 Gy/min at 240 kVp and 10 mA. Mice were irradiated in 75 cm<sup>2</sup> tissue culture flasks (BD Biosciences) depending on the experimental design.
immediately before irradiation and again 24 h later. Twenty-four hours after irradiation, 1×10^8 plaque-forming units (pfu) of Ad-RSV-Luc (or PBS) were injected intratumorally. Luc expression was quantitated by real-time bioluminescent imaging using the IVIS System 200 series (Xenogen Biosciences) at 24 h (baseline) and other time points (48, 120, and 192 h) after infection. D-Luciferin Firefly Potassium salt (15 mg/mL; Xenogen Biosciences) was administered at 150 mg/kg and imaging was performed after 5 min. One-minute continuous exposures were obtained at high sensitivity using the machine default settings (f/stop, 1; emission filter, open; field of view d, 12 cm; and subject height, 1.5 cm). The Xenogen software provides visual images of bioluminescence detection using a colored overlay on a photographic image taken immediately before luminescence measurements. The areas shown in color represent the regions of bioluminescence detection. Results were analyzed using Living Image 2.60.1 software (Xenogen Biosciences). Areas of Luc expression were defined using the autocontouring tool and the photon flux and average radiance (photons/cm^2/s) were estimated over the defined area.

Statistics. Statistical analysis was performed using Statistical Package for the Social Sciences software (SPSS, Inc.) package. For analysis of relative levels of in vivo Luc expression, data were compared using the Mann-Whitney U test.

Results

Radiation up-regulates GFP expression from replication-defective adenoviruses. The effect of radiation on gene expression from the CMV promoter in replication-defective adenoviral vectors expressing two different reporter gene constructs was initially assessed. In the case of Ad-CMV-GFP [multiplicity of infection (MOI), 1], a radiation dose-dependent increase in GFP expression was shown (Supplementary Figs. S1 and S2). Increased GFP expression was seen irrespective of the sequencing of radiation and virus infection, although a greater fold induction of GFP was seen when radiation was delivered before virus infection. Similar effects have been seen with Ad-CMV-lacZ, Ad-CMV-NIS, Ad-hTR-NIS, Ad-hTERT-NIS, and Ad-RSV-Luc. The lack of dependence on the sequencing of radiation and virus infection is suggestive of an effect of radiation that does not involve increased transduction of irradiated cells. Because the most important cell biological effect of radiation is to cause DNA damage, we examined the effect of radiation-induced DNA damage on virally mediated transgene expression.

Inhibition of repair of radiation-induced DNA damage up-regulates adenoviral-mediated expression of GFP. Initially, the effect of DNA repair inhibition with PARPi, ATMi, and DNA-PKi on the persistence of radiation-induced DSB at 24 hours was assessed in unirradiated and irradiated cells by FACS analysis for phospho-γH2AX foci. By 24 hours after radiation (5 Gy), the level of phospho-γH2AX foci detectable by FACS was essentially the same
as for control samples, consistent with rapid repair of radiation-induced DSB. In contrast, the levels of phospho-γH2AX foci relative to irradiated controls (no drug added) were markedly increased when cells were irradiated after exposure to PARPi (>5-fold increase), ATMi (>2-fold increase), and DNA-PKi (>4-fold increase) inhibitors (Fig. 1A and B). The relatively low level of persistence of phospho-γH2AX foci after exposure to ATMi may reflect the role of ATM in γH2AX phosphorylation. These findings were confirmed by confocal microscopy of HCT116 cells that showed maintenance of phospho-γH2AX foci after they were irradiated in the presence of DNA-PKi (Fig. 1C).

Thereafter, the effect of the DNA repair inhibitors on transgene expression from Ad-CMV-GFP was evaluated by flow cytometry and fluorescence microscopy. The individual DNA repair inhibitors had no effect on GFP expression in HCT116 cells that had not been exposed to irradiation (Fig. 2A). However, when these agents were used in irradiated cells, there was a 1.5- to 2-fold increase in the level of adenovirus-mediated GFP expression over and above that induced by irradiation alone (Fig. 2B; Supplementary Fig. S3). The effect was observed with all three inhibitors, although the greatest effect was observed with DNA-PKi. Subsequently, this effect was examined in a larger panel of five head and neck (SIHN-5B, HN-5, Cal-27, SIHN-011A, and SIHN-011B) and lung (H1299) cancer cells (Fig. 2C).

Having shown that inhibition of repair of radiation-induced DNA damage was associated with increased adenovirus-mediated gene expression, the time course of the effect was studied by varying the interval between irradiation and drug administration. DNA repair inhibitors were applied at different times (0, 4, 18, 24, and 48 hours) after radiation (5 Gy), and viral infections [Ad-CMV-GFP (MOI, 1)] were performed 24 hours after radiation. The effect of DNA repair inhibitors on transgene expression was most marked when the drugs were administered in close temporal proximity to the delivery of radiation (Fig. 2D).

Taken together, these data show that treating cells with PARPi, ATMi, and DNA-PKi close to the time of irradiation caused...
persistence of DNA DSB and that this was associated with increased adenovirus-mediated gene expression.

Radiation-induced up-regulation of adenoviral gene expression can be modulated by inhibitors of cellular signal transduction pathways. The effect of inhibition of MAPK/ERK, PI3K, and p38/SAPK signaling pathways on the level of adenoviral gene expression was tested using a panel of specific small-molecule inhibitors (Fig. 3A). MAPK/ERK (PD980059 and U0126) and PI3K (LY294002) inhibitors caused a significant reduction in the radiation-induced up-regulation of expression of GFP from Ad-CMV-GFP (MOI, 0.1). Reduction in radiation-induced up-regulation of GFP expression was seen after treatment with MAPK/ERK and PI3K inhibitors but not the p38/SAPK inhibitor. B and C, PD980059 showed dose-dependent inhibition of GFP expression from Ad-CMV-GFP (MOI, 0.1). There was no effect at 10 μmol/L PD980059 but a progressively greater effect at 25 and 50 μmol/L. Data are representative of at least three repeat experiments.

Figure 3. The MAPK/ERK and PI3K pathways are involved in radiation-induced up-regulation of adenoviral gene expression. A, HCT116 cells were infected with Ad-CMV-GFP (MOI, 0.1) and FACS analysis for GFP was performed at 72 h in (i) unirradiated cells (green shading) or irradiated (5 Gy) cells (pink line), (ii) irradiated (5 Gy) cells in the absence (green shading) or presence (blue line) of the MAPK/ERK inhibitor (PD980059), (iii) irradiated (5 Gy) cells in the absence (green shading) or presence (yellow line) of the MAPK/ERK inhibitor (U0126), (iv) irradiated (5 Gy) cells in the absence (green shading) or presence (orange line) of the p38/SAPK inhibitor (SB202190), and (v) irradiated (5 Gy) cells in the absence (green shading) or presence (green line) of the PI3K inhibitor (LY294002). Reduction in radiation-induced up-regulation of GFP expression was seen after treatment with MAPK/ERK and PI3K inhibitors but not the p38/SAPK inhibitor. B and C, PD980059 showed dose-dependent inhibition of GFP expression from Ad-CMV-GFP (MOI, 0.1). There was no effect at 10 μmol/L PD980059 but a progressively greater effect at 25 and 50 μmol/L. Data are representative of at least three repeat experiments.

The effect of DNA repair inhibition on gene expression is mediated through the Egr-1 promoter. The effect of small-molecule inhibitors of MAPK/ERK in reducing radiation-induced up-regulation of GFP expression from Ad-CMV-GFP suggests that this pathway is important in setting the level of gene expression from adenoviral vectors. Because radiation induces multiple transcription factors through activation of the MAPK/ERK pathway, we hypothesized that maintenance of unrepaired DNA damage may exert an effect through the MAPK/ERK pathway to alter the environment of the cell in favor of gene expression from adenoviral vectors. Egr-1, a transcription factor that forms part of the family of early growth response genes, is induced by radiation in a MAPK/ERK-dependent process (19, 20, 26, 27). Therefore, we hypothesized that maintenance of unrepaired DNA damage would enhance reporter gene expression from a plasmid construct regulated by the radioinducible synthetic E9 promoter. Therefore, HCT116 cells were transfected with pCMV-GFP or pE9-GFP plasmid constructs and irradiated in the presence (or absence) of DNA repair inhibitors. GFP expression from both pCMV-GFP and pE9-GFP constructs was enhanced by irradiation to 5 Gy (Fig. 4A and C). However, the DNA repair inhibitors only enhanced transgene expression in cells that had been transfected with pE9-GFP (Fig. 4C). PARPi, ATMi, and DNA-PKi had no effect on GFP expression from pE9-GFP in the absence of radiation (Fig. 4B).
DNA-PK inhibition up-regulates and maintains Ad-RSV-Luc expression in vivo. The effect of DNA repair inhibition on Luc gene expression was measured in vivo in HCT116 tumor-bearing mice. Animals were divided into three treatment groups (Ad-RSV-Luc alone, Ad-RSV-Luc plus 5 Gy, and Ad-RSV-Luc plus 5 Gy and DNA-PKI) and imaged using the IVIS System. Both of the 5 Gy and 5 Gy plus DNA-PKI groups showed significantly greater levels of Luc expression ($P < 0.05$) than the virus alone controls at all time points (Fig. 5A and B). Comparison between the Ad-RSV-Luc plus 5 Gy and the Ad-RSV-Luc plus 5 Gy and DNA-PKI groups revealed statistically significant maintenance of gene expression in vivo at 192 hours for animals who received the DNA-PKI ($P = 0.032$).

Discussion

Previous studies have reported that irradiation can increase cellular transduction and gene expression by adenoviral vectors. As part of this analysis, we have recently shown that the effect of radiation on viral gene expression cannot be explained solely in terms of increased viral infectivity (28). Instead, we have shown that cellular effects other than those related to increased expression of CAR and integrin are responsible for the enhanced levels of expression of several transgenes (GFP, Luc, lacZ, and sodium iodide symporter) from both constitutive viral (CMV and RSV) and tumor-specific promoters (hTR and hTERT). As a consequence, we hypothesized that radiation-induced changes in the intracellular signaling environment are responsible for mediating increased levels of gene expression from adenoviral vectors. In the studies reported here, we provide a mechanism by which this process may operate.

We have shown that specific inhibitors of components of the DNA repair apparatus are capable of maintaining radiation-induced DNA DSBs for up to 24 hours (Fig. 1A–C). The use of these agents on their own had no effect on adenovirus-mediated reporter gene expression (Fig. 2A). In combination with radiation-induced DNA damage, they further enhanced the level of GFP expression over and above that seen with radiation alone (Fig. 2B). Analysis of the DNA-PKI revealed that this effect operated in a panel of six different tumor cell lines (Fig. 2C). Studies using small-molecule inhibitors of signal transduction pathways implicated the MAPK/ERK and PI3K pathways in the radiation-induced up-regulation of Ad-CMV-GFP gene expression (Fig. 3A–C) as evidenced by reduced GFP expression in response to co-incubation with PD980059, U0126, and LY294002. Interestingly, the p38 MAPK inhibitor SB202190 had no effect on the effect of radiation on adenovirus-mediated gene expression. This finding is in variance with the recent report of the effect of radiation on gene expression in a pancreatic tumor cell line (14) and raises the possibility that the specific pathways involved in up-regulating adenoviral gene expression may be cell line dependent. In subsequent experiments using GFP-expressing reporter plasmids, we showed that maintenance of DNA damage by PARPi, ATMi, and DNA-PKi was associated with increased GFP expression from a radioinducible E9 promoter (Fig. 4C) but not from a constitutive viral CMV promoter (Fig. 4A). Taken together, these findings suggest that radiation-induced DSBs (and their maintenance) activate the MAPK/ERK/Egr-1 signaling pathway, which is able to alter the intracellular environment in such a way to favor adenoviral gene expression. It is also possible that reactive oxygen species generated during the radiation exposure are contributing to the process. Finally, the effect of DNA-PKI on in vivo gene expression was also shown using noninvasive imaging. Treatment with DNA-PKI was associated with maintenance of Luc expression to a statistically significant level at 8 days (Fig. 5A and B).

These data have important implications for therapeutic strategies in which radiation and adenoviral gene delivery are combined. Appropriate scheduling of the individual therapeutic components would offer the prospect of exploiting several advantageous combinatorial effects: (a) increased viral transduction of irradiated tumor cells, (b) increased absolute levels of radiation-induced and DNA repair inhibitor–induced up-regulation of gene expression in transduced cells, (c) maintenance of expression of therapeutic genes over a prolonged period (this would have specific relevance during fractionated radiotherapy), and (d) enhancement of radiation-induced cytotoxicity in transduced cancer cells by the DNA repair inhibitors. Ongoing experiments in our laboratory are examining the therapeutic effects of...
combining adenoviral gene delivery, DNA repair inhibition, and external beam or radioisotopic radiation delivery.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 5/23/2008; revised 8/8/2008; accepted 8/11/2008.

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We thank Dr. Graeme Smith (KuDOS Pharmaceuticals, Ltd.) for useful discussions.

References


Figure 5. Radiation and DNA repair inhibition increases Luc expression in vivo. Data represent the pooled results from three individual experiments. A, mice bearing HCT116 xenograft tumors were divided into three groups: Ad-Luc (0 Gy, 1 × 10⁸ pfu Ad-Luc, no DNA-PKi; n = 14), Ad-Luc + RT (5 Gy, 1 × 10⁸ pfu Ad-Luc, no DNA-PKi; n = 11), and Ad-Luc + RT + DNA-PKi (5 Gy, 1 × 10⁸ pfu Ad-Luc, DNA-PKi; n = 12). Ad-Luc was injected intratumorally 24 h after irradiation (0 or 5 Gy). Luc expression was quantitated by IVIS camera at 24, 48, 120, and 192 h after injection. Sequential images from representative mice from each of the three treatment groups show increased gene expression in the irradiated mice that were treated with DNA-PKi. B, Luc expression measured as mean photon flux in the three treatment groups. Levels in both irradiated groups were significantly greater than Ad-Luc only controls (P < 0.05). In irradiated animals treated with DNA-PKi, levels of Luc expression were greater than in those animals that had received radiation only. This difference was statistically significant at 192 h (P = 0.032).


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