Nitric Oxide and Ionizing Radiation Synergistically Promote Apoptosis and Growth Inhibition of Cancer by Activating p53

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ABSTRACT

Nitric oxide (NO) is a potent tumor radiosensitizer; however, its clinical use is limited by systemic side effects. We have demonstrated previously that gene transfer of the human inducible NO synthase (iNOS) gene into tumor cells and tumors induces high-output NO production that significantly enhances tumor radiosensitiveness, with no observed side effects. Notably, iNOS gene transfer enhances tumor radioresponsiveness via apoptotic cell death. Because NO and ionizing radiation are both known to promote p53-dependent apoptosis, we hypothesized that p53 activation might be a primary mechanism for the synergy of these two genotoxic stresses. We report that NO and ionizing radiation synergistically activate p53 in colorectal cancers grown in athymic mice by augmenting phosphorylation of p53 at serine 15. The effect of NO and ionizing radiation on tumor cell apoptosis and tumor radioresponsiveness is significantly reduced in p53 knockout isogenic cancer cell lines. Furthermore, the transfer of both p53 and iNOS genes into tumor cells lacking functional p53 enhanced their radioresponsiveness more than transfer of either gene alone.

INTRODUCTION

Ionizing radiation is integral to the treatment of many malignancies including colorectal, breast, lung, prostate, and esophageal cancers. Radiation is particularly important as an initial therapy for rectal cancer. In rectal cancer, the surgeon’s ability to obtain tumor clearance is often challenged by the anatomic constraints of a large tumor in a narrow pelvis. Radiation has the potential to decrease tumor size before surgery, enabling a greater chance of obtaining a tumor-free surgical margin, and can enable patients previously deemed unresectable to undergo curative surgical resection (1). Unfortunately, many tumors, including 21% to 62% of rectal cancers, are radioresistant (2–8).

Tumor hypoxia is a primary reason for radiation failure. Oxygen is the most potent radiosensitizer; however, approximately one third of human tumors have significant areas of hypoxia (9, 10), and the degree of hypoxia correlates well with the response to radiation (10). To circumvent this resistance mechanism, classes of drugs known as hypoxic cell radiosensitizers are in development. One of the most promising of these drugs is tirapazamine, a prodrug that is bioactivated under hypoxic conditions to generate a hydroxyl radical leading to DNA damage (11). Nitric oxide (NO) is an alternative hypoxic cell radiosensitizer that has also shown great clinical potential. NO is a highly reactive electrophile that is cytotoxic in the absence of oxygen. It is the second most potent chemical radiosensitizer (12, 13). NO diffuses readily from cell to cell, thus allowing it to potentially penetrate and exert its effects deep within a tumor mass, the so-called “bystander effect.” As a result, a large tumoricidal effect may occur even when only a minority of cells, for example, tumor-infiltrating macrophages, produce NO (14).

Multiple studies using drugs that release NO (NO donors) demonstrate that hypoxic tumor cells in vitro are significantly sensitized to irradiation (15–17). Initial enthusiasm for the use of NO donors has been dampened by the fact that in vivo administration of these agents may induce life-threatening systemic hypotension (18). Potential strategies to minimize the unwanted side effects of NO while maintaining the salutary tumoricidal effects of NO include the following: (a) the use of NO donors that are selectively activated in hypoxic regions and (b) up-regulation of inducible NO synthase (iNOS) gene expression within the tumor to increase localized NO production at the site where it can synergize with radiation (19, 20).

The efficacy and safety of intratumoral injection of the iNOS gene have been documented in several preclinical studies. Direct intratumoral injection of iNOS cDNA led to decreased tumor growth and necrosis in a thyroid cancer model (21). Tumor cell growth was inhibited with a retroviral vector engineered to overexpress iNOS selectively in carcinoembryonic antigen-producing tumor cells (22). Intratumoral injection of human kidney cells overexpressing iNOS significantly delayed colon and ovarian cancer growth associated with increased apoptosis (23). The efficacy of delivering the iNOS gene to radiosensitize tumors in vivo was first reported in a murine sarcoma model with injection of iNOS cDNA (24). Using an adenoviral vector carrying iNOS (AdiNOS), we have subsequently demonstrated the efficacy of iNOS gene therapy in radiosensitizing human colorectal cancer cell lines and tumors (20, 25). We have further demonstrated that iNOS overexpression by gene delivery enhances the radioresponsiveness of colorectal cancer via a caspase-dependent apoptotic mechanism (20, 25).

The primary objective of the present study was to determine the role of p53 in modulating the enhanced tumor cell apoptosis and tumor radioresponsiveness induced by NO and iNOS overexpression. Because NO and ionizing radiation are both known to induce a signal cascade leading to activation of p53 (26), we hypothesized that p53 activation might be a primary mechanism for the synergy of these two genotoxic stresses. We focused our studies on the p53 isoform (p53 phosphoserine-15p) activated by phosphorylation of serine 15 because it is felt to be critical in regulating the apoptotic response to DNA damage induced by either NO or ionizing radiation (26–29). Our secondary aim was to determine the effects of AdiNOS on the growth and radioresponsiveness of colorectal cancers lacking functional p53 because NO may potentially promote the growth of p53-mutated tumors (30).

MATERIALS AND METHODS

Cell Lines. The SNU-1040 cell line was obtained from the Korean Cell Bank (Seoul, Korea), and HCT-116 cell line was obtained from American Type Culture Collection (Manassas, VA). HCT-116 cells are primary colorectal adenocarcinoma cells that express wild-type (WT) p53. HCT-116 cells that were deficient in p53 [p53 knockout (KO)] by genetic engineering using homologous recombination were the kind gift of Bert Vogelstein (Johns Hopkins Medical School, Baltimore, MD). All cells were maintained in Dul-
DMEM, and then adding DMEM diluted in Optimum solution (Invitrogen, Carlsbad, CA). After 4 hours, the p53-phosphoserine 15 (Cell Signaling, Beverly, MA). For measurements of membrane was stained with Ponceau S (Sigma) to assure equal transfer of um-137 source; model 68 mark I small animal irradiator; JL Shepherd and cell density with cells in single cell suspension. Cells were irradiated with interpreted by the percentage of total cells appearing in each quadrant. Scan (Model 81831; Becton Dickinson, Mansfield, MA; ref. 33). Results were V-fluorescein isothiocyanate and propidium iodide and analyzed using FAC-apoptosis by the terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) technique as described by the Apoptosis Detection System (Promega, Madison, WI). Detection of localized green fluorescent apoptotic cells (fluorescein 12-dUTP) in a red background (propidium iodide) was performed by fluorescence microscopy (25). The apoptotic fraction was determined in a blinded fashion by manually counting the frequency of green and red fluorescent cells per 5 to 10 random high-power fields (HPFs (magnification, ×20)) for each tumor section (4–6 sections per tumor).

**Immunochemistry.** Tumors were sectioned (5-μm thick) and stained for p53-phosphoserine 15. Sections were incubated with a polyclonal anti-human p53-phosphoserine 15 antibody (Cell Signaling) for 1 hour, washed in bovine serum albumin (BSA), incubated with goat antirabbit cy3 (Jackson Immuno Research Laboratories, West Grove, PA) for 1 hour, and then stained with Hoechst dye (Sigma) and mounted. Slides were examined using an Olympus BX 51 fluorescence microscope. The frequency of p53-phosphoserine 15-positive cells was determined in a blinded fashion by manually counting the frequency of red fluorescent cells per 5 to 10 random HPFs (magnification, ×20) for each tumor section (4–6 sections per tumor).

For costaining for p53-phosphoserine 15 and INOS, tumor sections were blocked in normal goat serum (Sigma), incubated with an INOS rabbit polyclonal antibody IgG (1:500; Transduction Laboratories, Lexington, KY) for 1 hour, and washed in BSA. Sections were then incubated with cy3-conjugated goat antirabbit antibody fragment 1 fragments (Jackson Immuno Research Laboratories) for 1 hour, washed in BSA, and incubated with a polyclonal antihuman p53-phosphoserine 15 antibody (Cell Signaling) for 1 hour. Sections were then washed with BSA, incubated with goat antirabbit Alexa 488 (Molecular Probes, Eugene, OR) for 1 hour, washed again with BSA, stained with Hoechst, and mounted. For costaining for p53-phosphoserine 15 and TUNEL, tumor sections were stained for TUNEL as described, blocked in normal goat serum (Sigma), incubated with a polyclonal antihuman p53-phosphoserine 15 antibody (Cell Signaling) for 1 hour, stained with Hoechst, and mounted.

**In vivo Radiation Experiments.** Athymic nude mice (Harlan Laboratories, Indianapolis, IN) received injection with 1 × 10⁶ tumor cells in the right hind limb. Tumor size was measured daily in two dimensions using Vernier calipers. When tumors reached 3 to 4 mm in diameter, tumor volume (V) was calculated according to the following equation: V = (π/6) (mean diameter)³. Tumor size was measured by flow cytometry using the annexin V-fluorescein isothiocyanate Apoptosis Detection Kit (BD Bioscience, San Diego, CA), as previously described (20). At 72 hours after irradiation, cells were incubated with annexin V-fluorescein isothiocyanate and propidium iodide and analyzed using FAC-Scan (Model 81831; Becton Dickinson, Mansfield, MA; ref. 33). Results were interpreted by the percentage of total cells appearing in each quadrant.

**Measurement of Apoptosis by Flow Cytometry.** Apoptosis was determined by flow cytometry using the annexin V-fluorescein isothiocyanate Apoptosis Detection Kit (BD Bioscience, San Diego, CA), as previously described (20). At 72 hours after irradiation, cells were incubated with annexin V-fluorescein isothiocyanate and propidium iodide and analyzed using FAC-Scan (Model 81831; Becton Dickinson, Mansfield, MA; ref. 33). Results were interpreted by the percentage of total cells appearing in each quadrant.

**Clonogenic Assay.** Cell survival was determined by clonogenic assay, as described previously (25). Briefly, aerobic irradiations were performed at low cell density with cells in single cell suspension. Cells were irradiated with graded doses (0–12 Gy) of external beam irradiation (80.8 rads/minute, cesium-137 source; model 68 mark I small animal irradiator; JL Shepherd and Associates). After irradiation, cells were plated in colony dishes (n = 4 wells per group) at variable density, depending on the radiation dose given (100–100,000 cells per plate). Plating efficiency ranged from 60% to 89%. Formed colonies (≥50 cells) were manually counted at 10 to 14 days. The plating efficiency was calculated as follows: plating efficiency = (number of colonies counted/number of cells seeded) × 100. The surviving fraction was determined as follows: surviving fraction = (number of colonies)/(number of cells seeded × plating efficiency/100). Surviving fractions were calculated using the FIT program (version 2) for analysis of cellular survival data (developed by Dr. N. Albright, Department of Radiation Oncology, University of California at San Francisco, San Francisco CA).
TCC→TTC base substitution) in the p53 gene localized to codon 241 of exon 7. This specific point mutation renders p53 protein nonfunctional (26). To determine whether the p53 signaling pathways were intact in SNU-1040 cells despite having a nonfunctional p53 gene, p21\textsuperscript{waf1} protein expression and apoptosis were determined after infecting cells with an adenovirus carrying the WT 53 gene (data not shown). Infection of cells with Adp53 led to expression of WT p53 and subsequent p21\textsuperscript{waf1} expression. Expression of neither p53 nor p21 was detectable in untransfected SNU-1040 cells. Expression of p53 resulted in increased apoptosis. Apoptosis occurred in 30% to 40% of cells at 30 hours after gene transduction, with the majority of cells (80–90%) in late apoptosis (positive for both annexin V and propidium iodide) by 48 hours, indicating progression of this p53-dependent cell death pathway. P53 status was also confirmed in HCT-116 cells and p53 KO cells by Western blotting (data not shown).

**AdiNOS + Radiation Induces Activation of p53 in Tumors.** We have demonstrated previously (25) that iNOS overexpression in HCT-116 tumors enhances tumor radioresponsiveness by promoting apoptosis. NO is known to up-regulate and activate p53 (26). Activation of p53 principally through phosphorylation of p53 at serine 15 also accounts for the p53-dependent apoptotic response to DNA damage (28). We hypothesized that phosphorylation of p53 at serine 15 would be a major mechanism for the synergistic cytoxic effects of NO and ionizing irradiation on tumors expressing WT p53.

We examined p53 activation in *in vivo* grown p53 WT tumors (HCT-116) treated with AdiNOS + radiotherapy (RT). HCT-116 tumors received injection for 2 consecutive days with either AdiNOS or Ad-CTL or received no injection. Adenovirus was injected for 2 consecutive days using equivalent volumes (20 L/d) and equivalent total number of viral particles (9.0 \times 10^7 plaque-forming units). Tumors were irradiated with a single fraction of 2 Gy on the 3rd consecutive day and harvested 24 hours later. Expression of p53-phosphoserine 15 was quantified (percentage of positively stained cells per 10 HPF) in tumors (*n* = 4 per group). Tumors treated with AdiNOS + RT had the greatest (*P < 0.001) percentage of cells with
activated p53 compared with all other groups (control, untreated, irradiated, and Ad-CTL + radiation; Fig. 1A–D). AdiNOS + RT caused a significant \( (P = 0.001) \) 55-fold increase in the percentage of cells (mean ± SE) with activated p53 with a median of 11.1 ± 3.1% of cells positive for AdiNOS + RT compared with 1.4 ± 0.3% cells positive with Ad-CTL + RT; 2.7 ± 0.4% cells positive with RT, and 0.2 ± 0.1% cells positive in untreated controls. Subcellular localization studies indicated that activated p53 was localized to the tumor cell nucleus (Fig. 1D).

**Activated p53 Is Expressed Predominantly in Apoptotic Tumor Regions.** To further confirm the role of NO and activated p53 in enhancing radiation-induced apoptosis in vivo, we examined the localization of activated p53 and iNOS expression relative to apoptotic areas in tumors that overexpress iNOS and had been irradiated. Activated p53 colocalized with iNOS expression in tumors treated with AdiNOS + RT, indicating a causal role for NO in enhancing p53 activation in vivo (Fig. 1E). Tumor apoptosis (TUNEL positivity) was closely associated with activated p53 expression (Fig. 1F) and iNOS expression (data not shown), indicating a causal role for p53-phosphoserine 15 and NO in enhancing radiation-induced apoptosis in vivo in colorectal tumors.

**Nitric Oxide and Irradiation Synergistically Increase p53 Expression and Activation in Tumor Cells.** To verify that p53 is activated synergistically by NO and ionizing radiation, we initially examined the effects of exogenous NO and ionizing radiation on WT p53 tumor cells in culture. Expression of p53 and activated p53 protein (p53-phosphoserine 15) was measured 6 hours after treatment of HCT-116 cells with SNAP, RT (1–2 Gy), or SNAP + RT (Fig. 2A and B). Cells were treated with the NO donor for 1 hour before irradiation, and oxidized SNAP was used as a control. Cells exposed to both NO and RT had the greatest expression of both p53 and activated p53 protein. The expression of p53 and activated p53 in cells treated with combined NO and RT was more than additive, indicating synergy for regulation at the transcriptional and posttranslational levels.

**Loss of p53 Abrogates the Enhanced Tumor Cell Radioresponsiveness Induced by Nitric Oxide.** Because NO enhanced tumor cell radiosensitivity with gene transfer of WT p53 to p53-mutated cells and associated with p53 activation in p53 WT cells, we examined whether loss of p53 in p53 KO cells would decrease radioreponsiveness induced by NO. The enhancing effects of NO on radiation-induced apoptosis were examined in p53 KO HCT-116 cells and simultaneously compared with the parental p53 WT cell line. The effect of NO combined with radiation on apoptosis was compared between both cell lines and quantified by flow cytometry (Fig. 2C). NO enhanced the effects of radiation in both cell lines. However, NO enhanced the effects of radiation on apoptosis to a greater extent in HCT-116 cells that expressed a functional p53 gene (7.2-fold in p53 WT cells versus 2.0-fold in p53 KO cells). These data are consistent with activated p53 mediating the enhanced radiation apoptosis induced by NO.

**Coordinate Gene Transfer of Inducible Nitric Oxide Synthase and p53 Synergistically Enhances the Cytotoxic Effects of Radiation on Clonogenic Survival.** To further examine the interaction between NO, p53, and radiation on cell survival, SNU-1040 cells were transduced with the iNOS and p53 genes, and clonogenic survival was measured with and without radiation (Fig. 2D). Cells were transduced with either varied multiplicities of infection (MOIs) of AdiNOS, iNOS, p53, and AdiNOS + p53, respectively, followed by radiation. Control cells were transduced with Ad-Control without either iNOS, p53, or both genes. The effects of each gene on radiation-induced apoptosis were determined by the clonogenic survival method. The data in Fig. 2D illustrate that the combination of NO and radiation has a greater effect on clonogenic survival in WT cells compared with p53 KO cells.

![Fig. 2](cancerres.aacrjournals.org)
Adp53, or both vectors. Transduction of cells with either iNOS or p53 genes significantly ($P < 0.001$) enhanced the tumoricidal effects of radiation alone. Transduction of both iNOS and p53 genes into cells produced a greater ($P < 0.001$) radiation enhancement than that accomplished by single gene transfer. Radiation combined with transduction of both genes led to a significantly ($P < 0.01$) decreased frequency of colonies compared with transduction of both genes in the absence of radiation. Coexpression of the iNOS and p53 genes led to significant ($P < 0.001$) synergistic effects (with or without radiation). In unirradiated cells, the expression of both genes led to a 31% mean decrease in the number of colonies formed, compared with 1% with either gene alone. In irradiated cells, coexpression of both iNOS and p53 genes decreased colonies by a mean of 53% compared with only 12% observed for either iNOS or p53 gene transfer alone.

**Enhanced Tumor Radiosensitivity Induced by AdiNOS Is Reduced by Deletion of p53 Expression.** To determine whether p53 modulates the enhanced radiosensitiveness of tumors overexpressing iNOS, tumors derived from p53 KO HCT-116 cells were infected with AdiNOS, irradiated, and compared with previous results obtained for p53 WT tumors (25). Forty-six mice bearing p53 KO tumors were randomized to the same six treatment groups ($n = 6–8$ mice per group) as WT p53 tumor-bearing mice: (a) no treatment (control); (b) radiation alone (2 Gy $\times 3$ fractions); (c) Ad-CTL injection; (d) AdiNOS injection; (e) Ad-CTL injection + radiation (2 Gy $\times 3$ fractions); and (f) AdiNOS injection + radiation (2 Gy $\times 3$ fractions). Radiation was initiated on the 2nd day of viral injection with 3 consecutive days of treatment with clinically relevant fractions of 2 Gy.

AdiNOS infection combined with radiation caused the greatest delay in tumor growth (Fig. 3A and B). Treatment of tumors with empty viral vector and radiation resulted in a similar delay in tumor growth as tumors treated with radiation alone. The tumor doubling time was significantly ($P < 0.001$) prolonged by 2.5-fold with AdiNOS + RT (Fig. 3B). The mean growth delay for tumors treated with AdiNOS + RT was 11.4 days, which was significantly ($P < 0.05$) greater than the growth delay for tumors treated with Ad-CTL + RT (7.2 days) or RT alone (6.7 days). The mean growth delay for tumors treated with AdiNOS + RT (11.4 days) was also greater than the combined mean growth delays of tumors treated with AdiNOS alone (1.8 days) or radiation alone (6.7 days). As a result, AdiNOS injection of p53 KO tumors caused a 1.34-fold radiopotentiation. The radiopotentiation induced by AdiNOS injection was less than the radiopotenti-ation (1.93-fold) observed previously in p53 WT tumors, indicating that p53 mediates, in part, the enhanced radioprotectiveness of tumors injected with AdiNOS. In contrast to the effect of AdiNOS and radiation on p53 WT tumors, AdiNOS and radiation did not induce tumor regression in p53 KO tumors, suggesting that the presence of WT p53 is critical for induction of tumor apoptosis. Furthermore, these studies indicate that p53 mediates the enhanced tumor radio-protectiveness induced by iNOS overexpression in colorectal cancer.

**DISCUSSION**

Apoptosis is a common mechanism of cell death accounting for the radiation enhancement observed with the use of many radiosensitizers in colorectal cancer. Apoptosis accounts for 60% of the decrease in clonogenic survival in HT-29 colorectal cancer cells treated with the radiosensitizer 2′,2′-difluoro-2′-deoxyuridine plus radiation (34). Similarly, apoptosis is a significant mechanism inherent to tumor cell death and delayed tumor growth in HCT-116 colon cancer cells treated with radiation and radiosensitizers (35, 36). We demonstrated previously (20) that iNOS overexpression increases radiation-induced apoptosis 2- and 4-fold in SNU-1040 and HCT-116 colon cancer cells, respectively. Additionally, apoptosis is an important mechanism associated with the enhanced radiation response of HCT-116 tumors treated with intratumoral injection of AdiNOS (25).

Our present work demonstrates that NO synergizes with ionizing irradiation to enhance tumor radiosensitivity via activation of p53. Overexpression of iNOS combined with radiation activated p53 intratumorally to a greater extent than radiation alone. Expression of p53 phosphoserine-15P in tumors treated with AdiNOS + RT occurred predominantly in areas rich in cells expressing iNOS, supporting the role of NO in p53 activation in vivo. Apoptosis was similarly increased in these same tumor areas, implicating NO and p53 activation as biological modifiers of the tumor response to iNOS gene therapy combined with radiation. Similarly, NO and RT synergistically activated p53, resulting in enhanced expression of p53 phosphoserine-15P in treated tumor cells.

We further examined the interaction of NO and p53 in modulating tumor cell radiosensitivity. Increasing NO production by iNOS gene transfer synergized with gene delivery of WT p53 to enhance overall radiosensitivity of p53-mutated cells. By using isogenic p53 KO cells, we further demonstrated that loss of p53 decreases the synergy of NO and radiation in mediating tumor cell apoptosis. Using p53 KO
NO + RT SYNERGISTICALLY ENHANCE TUMOR RESPONSIVENESS

tumors, we additionally confirmed that p53 modulates the radioenhancing effects of intratumoral delivery of iNOS, with the radioenhancing effects of iNOS gene delivery reduced in p53 KO tumors when compared with p53 WT tumors. Treatment of p53 WT tumors with combined iNOS gene delivery and radiation leads to considerable tumor regression associated with increased apoptosis (25). In contrast, tumor regression did not occur in p53 KO tumors treated with AdiNOS + RT. Taken together, these observations lead us to conclude that activation of the p53-dependent apoptotic pathway is a major mechanism involved in the tumor radiosensitivity induced by iNOS overexpression.

Activation of p53 may occur as a result of posttranslational protein modification via phosphorylation, acetylation, and ubiquitination of p53 (37). Phosphorylation of serine residues in the NH2 terminus of p53 inhibit nuclear export of p53 and increase p53 transcriptional activity (38). Phosphorylation of serine 15 in the NH2 terminus is a major molecular event leading to cellular apoptosis in response to DNA damage (28). Cells respond to both ionizing irradiation and NO by activating p53 via phosphorylation of serine 15; however, the signaling pathways leading to this response differ (26, 29, 37). Ionizing radiation induces phosphorylation of serine 15 via the ATM kinase, whereas phosphorylation induced by NO occurs principally via the ATR kinase. NO also stimulates DNA-dependent protein kinase and p38 mitogen-activated protein kinase, both of which may enhance the phosphorylation of serine 15. Because p53-phosphoserine 15 is a major regulator of the apoptotic response but is induced by unique kinase pathways in response to either NO or radiation, we hypothesized that phosphorylation of p53 at serine 15 might be the basis for the synergy of NO and radiation on tumor cell apoptosis and growth delay in vivo. Consistent with this hypothesis, we observed that NO and RT synergistically increased p53-phosphoserine 15 in tumor cells. Tumors treated with AdiNOS + RT also had increased p53-phosphoserine 15 that was localized to the nucleus. Future studies examining the combined effects of NO and RT on upstream kinases will help to define the specific mechanisms for the synergistic increase in p53 activation via phosphorylation.

Our KO studies demonstrate that p53 activation is a primary cause for the synergy between NO and radiation in inhibition of tumor growth and enhancement of apoptosis. We cannot, however, conclude from these studies that phosphorylation specifically at serine 15 of p53 is the specific or only cause for the observed cytotoxic synergy. Additional studies examining the effects of NO and RT in cells exhibiting point mutations at serine 15 of p53 (28) may help us to define the specific role of this p53 modification. Activation of p53 via phosphorylation of serine 20 and serine 46 may also be integrally involved in the response to NO and RT because p53 species modified at either of these positions promote cellular apoptosis (28, 37). Decreased nuclear export of p53 by inhibition of the interaction between p53 and mdm2 or by modification of the nuclear export signal at the COOH terminus of p53 may represent other potential mechanisms for the activation of p53 by NO and RT (37). Our work demonstrates that NO enhances the radiosensitivity of tumors by activation of the p53-dependent apoptotic program. Studies from our laboratory and others (39, 40) demonstrate in similar tumor models that NO and p53 interact to modulate DNA repair after radiation, suggesting that DNA repair may be another potential mechanism for the effects seen in our model.

Mutations in p53 are common in cancers of the colon, prostate, and head and neck and may lead to radiation resistance (41, 42). Gene transfer of the WT p53 gene into human colon and brain cancers enhances the effects of radiation in xenograft models (43, 44). We have shown previously (20, 25) that both iNOS gene transfer and addition of exogenous NO to p53-mutated colon cancer cells enhance radiation-induced apoptosis and overall clonogenic radiosensitivity. Although these studies demonstrate that NO can enhance tumor cell radiosensitivity via p53-independent pathways as well, we had not tested the efficacy of intratumoral injection of AdiNOS + RT in tumors lacking functional p53. These studies were also essential because prior work by others (30) demonstrated that isogenic clones of p53-mutated colon cancer cells that expressed increased iNOS grew faster in vivo. In contrast to these observations, we have found that intratumoral injection of AdiNOS alone had no growth-promoting effect. In addition, AdiNOS injection significantly enhanced tumor radiore sponsiveness in this model. Although we demonstrate that p53 plays a key role in modulating the response of tumors to NO and ionizing radiation, we also demonstrate that NO enhances the effects of radiation independent of p53. These results indicate that intratumoral injection of AdiNOS is a promising gene therapy to radiosensitize both p53 WT and p53-mutated colorectal cancers.

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