TGFB1 Inhibition Increases the Radiosensitivity of Breast Cancer Cells In Vitro and Promotes Tumor Control by Radiation In Vivo

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Abstract

**Purpose:** To determine whether inhibition of TGFB signaling prior to irradiation sensitizes human and murine cancer cells in vitro and in vivo.

**Experimental Design:** TGFB-mediated growth and Smad phosphorylation of MCF7, Hs578T, MDA-MB-231, and T47D human breast cancer cell lines were examined in both clonogenic survival and DNA damage response following graded radiation doses with and without pretreatment with LY364947, a small molecule inhibitor of the TGFB type I receptor kinase. The DNA damage response was assessed in irradiated MDA-MB-231 cells pretreated with LY364947 in vitro and LY2109761, a pharmacokinetically stable inhibitor of TGFB signaling, in vivo. The in vitro response of a syngeneic murine tumor, 4T1, was tested using a TGFB neutralizing antibody, 1D11, with single or fractionated radiation doses in vivo.

**Results:** Human breast cancer cell lines pretreated with TGFB small molecule inhibitor were radiosensitized, irrespective of sensitivity to TGFB growth inhibition. Consistent with increased clonogenic cell death, radiation-induced phosphorylation of H2AX and p53 was significantly reduced in MDA-MB-231 triple-negative breast cancer cells when pretreated in vitro or in vivo with a TGFB type I receptor kinase inhibitor. Moreover, TGFB neutralizing antibodies increased radiation sensitivity, blocked H2AX focus formation, and significantly increased tumor growth delay in 4T1 murine mammary tumors in response to single and fractionated radiation exposures.

**Conclusion:** These results show that TGFB inhibition prior to radiation attenuated DNA damage responses, increased clonogenic cell death, and promoted tumor growth delay, and may be an effective adjunct in cancer radiotherapy. Clin Cancer Res; 17(21); 6754–65. ©2011 AACR.

Introduction

Ionizing radiation is an effective cancer treatment modality that is administered in a manner that maximizes tumor damage while minimizing effects on normal adjacent tissue (1). Further benefit can be achieved through administration of drugs or biological agents that either promote tumor cytotoxicity or protect normal tissue from dose-limiting toxicity, such as fibrosis. TGFB is a candidate target whose inhibition could potentially do both.

There is substantial evidence that TGFB plays a crucial role in the response to ionizing radiation (2). TGFB is a pleiotropic cytokine that is important in normal tissue homeostasis, regulates inflammation and immune responses, and suppresses epithelial proliferation. TGFB is activated in irradiated tissues, presumably because the latent TGFB complex has a specific redox-sensitive conformation activated by reactive oxygen species, which are generated by radiation (3). Some preclinical models suggest that radiation-induced TGFB contribute to metastasis (4); consistent with this irradiated cells are primed to undergo TGFB-mediated epithelial-mesenchymal transition that increases motility and invasion (5, 6). Radiation-induced TGFB activity can also be sustained beyond an acute response, which may drive function-compromising fibrosis, a common sequel following radiotherapy, in susceptible tissues (7–11). Significant experimental support for a critical role of TGFB in radiation-induced fibrosis is provided by studies in which blocking TGFB production or signaling significantly reduces fibrosis in preclinical rodent models (8, 9, 12–15). This has led to recognition that TGFB inhibition following radiotherapy could prevent normal tissue toxicity due to fibrosis (9, 11, 16), although this concept awaits testing in clinical trials.
Radiosensitization by TGFβ Inhibitors

Translational Relevance

Radiation therapy is an important treatment modality for breast and other cancers. We have shown that ionizing radiation induces TGFβ activation and that TGFβ inhibition compromises canonical radiation responses in epithelial cells via inhibition of ataxia telangiectasia mutated kinase activity. We hypothesized that inhibiting TGFβ during radiotherapy could increase tumor response to radiation therapy if cancer cells maintain TGFβ regulation of the DNA damage response. We show that human and mouse breast cancer cells line were radiosensitized when pretreated with either a small molecule inhibitor of the TGFβ type I receptor kinase or pan-specific TGFβ neutralizing antibody. Consistent with this, phosphorylation of γH2AX and other DNA damage responses were significantly reduced by TGFβ inhibition prior to \textit{in vitro} or \textit{in vivo} irradiation. Moreover, tumor growth delay following radiation was significantly greater when TGFβ neutralizing antibodies were administered before either single or multiple radiation fractions. These preclinical studies suggest that TGFβ inhibition during radiotherapy could provide significant benefit.

A new role for TGFβ in mediating the rapid execution of the DNA damage response (DDR) has been identified (reviewed in ref. 2). Perhaps the first indication of its critical role was the observation that epithelial tissues of \textit{Tgfb}\textsubscript{1} heterozygote embryos exposed \textit{in utero} to 5 Gy showed significantly less radiation-induced apoptosis and \textit{Tgfb}\textsubscript{1} null embryos fail to undergo either apoptosis or inhibition of cell cycle (17). TGFβ depletion by gene knockout or transient depletion by TGFβ neutralizing antibody also reduced radiation-induced p53 phosphorylation \textit{in vivo} (17). Subsequent studies by Boothman and colleagues showed that TGFβ initiates a radiation survival mechanism dependent on secretory clusterin (18). However, the failure of the proximal DDR is attributed to compromised ataxia telangiectasia mutated (ATM) protein auto-phosphorylation and kinase activity, which decreases phosphorylation of critical DNA damage transducers γH2AX, Chk2, p53, and Rad17 and in turn, abrogates cell fate decisions (19). As a consequence, both \textit{Tgfb}\textsubscript{1} null murine epithelial cells and human cells in which TGFβ signaling is pharmacologically blocked are more radiosensitive, as measured by clonogenic survival (19). Furthermore, Rodemann and colleagues showed that TGFβ antisense also compromises ATM kinase-dependent phosphorylations in irradiated A549 lung cancer cells (20). ATM is a nuclear sensor of DNA damage that initiates, recruits, and activates a complex program of checkpoints for cell cycle, apoptosis, and genomic integrity and its loss or inhibition enhances radiosensitivity (see reviews in refs. 21, 22). As a consequence, radiosensitivity, as measured by clonogenic survival, increases in both murine epithelial cells from \textit{Tgfb}\textsubscript{1} null mice and in human epithelial cells in which TGFβ signaling is pharmacologically blocked (19).

Most solid cancers escape TGFβ growth regulation and amplify TGFβ production, which in turn suppresses immnosurveillance and enhances invasion and metastasis (23), thus leading to a protumorigenic environment. TGFβ also compromises responses to chemotherapy (reviewed in ref. 24), by mechanism that remains undefined. Taken together, increased levels of TGFβ protein in cancer, TGFβ activation by radiation, and TGFβ regulation of ATM kinase activity and DDR, suggest that TGFβ could protect cancer cells from DNA damage, thus decreasing the efficacy of radiotherapy. A small molecule inhibitor of TGFβ signaling has shown efficacy in combination with radiation and chemotherapy in human glioblastoma xenografts (25). However, breast cancer often evades the growth-inhibitory action of TGFβ by selectively eliminating cytostatic gene responses due to attenuated C/EBPβ transcriptional control (26). Although TGFβ control of ATM is not dependent on cell-cycle status per se in normal cells (19), it is unknown whether TGFβ inhibition can increase radiosensitivity when growth regulation is truncated in breast cancer cells. Thus, to assess the therapeutic potential of TGFβ inhibition in radiotherapy for breast cancer, we determined the relationship between sensitivity to TGFβ-mediated growth inhibition, molecular responses to radiation, and radiosensitivity in human and murine breast cancer cells. This study shows that TGFβ inhibition increases radiosensitivity of breast cancer cells irrespective of growth sensitivity, compromises DDR, and promotes radiation-induced tumor growth delay.

Materials and Methods

Cell culture

Human breast cancer cell lines MCF10A, MCF7, MDA-MB-231, T47D and Hs578T cells, and murine breast cancer cell line 4T1 were obtained from American Type Culture Collection and were cultured in MEGM, 10% FBS-MEME, 10% FBS-DMEM, 10% FBS-DMEM and 10% FBS-DMEM media, respectively, at 37°C with 5% CO₂. Cells were treated in 10% serum replacement medium (SRM; Knockout SR, Life Technologies, Inc.) containing either 500 pg/mL TGFβ (R&D Systems), 400 nmol/L small molecule inhibitor of the TGFβ type I receptor kinase, LY364947 ([3-(Pyridin-2-yl)-4-(4-quinonyl)]-1H-pyrazole); Lilly designation HTS466284; Catalogue no. 616451, Calbiochem) or 1D11, a pan-isofrom, neutralizing TGFβ monoclonal antibody or 13C4, murine monoclonal isotype control antibody (Genzyme). For growth studies, cells were trypsinized and counted using a Coulter counter at 24-hour and 48-hour posttreatment. Cells were grown in complete media for 48 hours, followed by LY364947 treatment (400 nmol/L) in 10% SRM for 24 hours prior to irradiation with 2 Gy for γH2AX foci induction by immunofluorescence and 5 Gy for DDR using immunoblotting.

Colony assay

Human breast cancer cells grown for 48 hours to 70% confluence were treated with 400 nmol/L of LY364947...
kinase inhibitor for 48 hours before and 3-hour postradiation exposure with differential doses using a 250-kVp X-ray (0.61 Gy/min). Murine mammary 4T1 tumor cells were treated with 1D11 or 13C4 for 24 hours before irradiation to the indicated dose with Clinac 2300 C/D linear accelerator. Cells were trypsinized 3-hour postirradiation and were plated in triplicates at 3 dilutions into 6-well cell culture plates in serum containing media. Cells were allowed to grow for 10 to 12 days followed by fixing and staining with crystal violet. Colonies containing more than 50 cells were counted. To determine percent survival, colony forming efficiency was determined, averaged, and normalized to those of the nonirradiated control. For each radiation dose, the mean number of colonies obtained from 3 wells were corrected according to plating efficiency and used to calculate the cell survival at each dose (27). The significance of the difference between the dose responses was calculated by conducting a 1-way ANOVA test. We chose the hierarchical ANOVA models to control the heterogeneity of the data caused by the interaction of the 2 variables and to infer the statistical significant differences in the clonogenic survival between treatments for the given radiation doses using factorial ANOVAs followed by least significant difference 2-by-2 comparisons.

Immunoblot analysis

To examine molecular responses to TGFβ or radiation, 7 × 10^5 cells were grown in complete media for 48 hours, followed by LY364947 treatment (400 nmol/L) in 10% SRM for 24 hours followed by exposure to 5 Gy, which were lysed after 1 hour, or treated with 500 pg/mL TGFβ, which were lysed after 30 minutes. The extracts were subjected to immunoblot analysis with one of the following primary antibodies: Smad2/3 at 1:500 (Catalogue no. 11858, Cell Signaling), Smad2/3 at 1:500 (Catalogue no. 610842, BD Transduction Laboratories), p53 serine 15 phosphorylation at 1:500 (Catalogue no. 92845, Cell Signaling), p53 serine 20 phosphorylation at 1:500 (Catalogue no. 92879, Cell Signaling), and p53 at 1:500 (Clone DO-7 + BP53-12, Catalogue no. MS-738-P0 Neomarkers). Protein estimation was carried out using the BCA protein assay kit from Pierce. One hundred micrograms of protein was electrophoresed on a 4% to 15% gradient gel of protein was electrophoresed on a 4% to 15% gradient gel of protein was electrophoresed on a 4% to 15% gradient gel from 3 wells were corrected according to plating efficiency and used to calculate the cell survival at each dose (27). The significance of the difference between the dose responses was calculated by conducting a 1-way ANOVA test. We chose the hierarchical ANOVA models to control the heterogeneity of the data caused by the interaction of the 2 variables and to infer the statistical significant differences in the clonogenic survival between treatments for the given radiation doses using factorial ANOVAs followed by least significant difference 2-by-2 comparisons.

In vivo tumor studies

All animal studies were conducted using protocols that had undergone appropriate review and approval at each institution. Severe combined immunodeficient mice/beige mice were injected subcutaneously at the UCSF Preclinical Therapeutics Core with 2 × 10^6 MDA-MB-231 breast cancer cells on each flank at the UCSF Preclinical Therapeutics Core. LY2109761 (100 mg/kg, Eli Lilly & Co.; ref. 28) or vehicle control was administered once by oral gavage at day 29 when tumors were approximately 0.8 cm in diameter. Two hours later, the mice were irradiated or mock irradiated using a medial exposure body shield with 3 Gy Cs-radiation source. Tumors (n = 3 per treatment) were excised 1 hour after radiation exposure and frozen in OCT using dry ice/ethanol bath. Balb/C mice were injected subcutaneously with 5 × 10^4 4T1 cells on left flank of the mice at NYU Medical Center and were treated at day 13, upon reaching approximately 100 mm^3. Neutralizing antibody 1D11 or isotype control, 13C4 (29), were administered by intraperitoneally (i.p.) injection (5 or 50 mg/kg, kindly provided by Genzyme) 24 hours before tumors were locally irradiated with a single dose of 8 Gy or 3 doses of 12 Gy on 3 consecutive days. Briefly, mice were lightly anesthetized by i.p. injection of Avertin (240 mg/kg) and then positioned on a dedicated plexiglass tray to irradiate a field including the tumor with 5-mm margins using a Clinac 2300 C/D linear accelerator (Varian Medical Systems) fitted with a 25-mm radiosurgery conical collimator (BrainLAB AG). Superflab bolus (1.5 cm tissue equivalent material) was placed over the tumor, and a source-to-skin distance of 100 cm was set. Radiation was delivered at 600 cGy/min with 6 MV X-rays. Mice were monitored thrice weekly for signs of toxicity and tumors volumes were measured with a caliper. Tumors volumes were calculated as length × width^2 × 0.52.

Statistical analysis

Random coefficients regression was used to assess the effect of treatment on log tumor growth; log volumes were used in place of observed volumes because the change in log volume over the course of follow-up was well
approximated as linear. The model to predict log tumor volume included elapsed time from baseline (defined as first day of measurement) as a numeric factor, treatment as a classification factor and the term representing the interaction of treatment with time. The interaction term was partitioned to derive tests that compared treatment arms in terms of tumor growth rate. The correlation structure imparted by the inclusion of multiple-dependent variable observations per animal was modeled by assuming observations to be correlated only when acquired from the same animal with the strength of correlation between observations inversely dependent on the elapsed time between observations (i.e., measures are more strongly correlated when taken closer together in time).

After conducting a Shapiro–Wilks test to verify the Gaussian distribution of the data, an ANOVA was used to compare treatment arms in terms of the normalized tumor volumes or tumor weight observed at each day of measurement. The error variance was allowed to differ across treatment arms to avoid the unnecessary assumption of variance homogeneity. All reported P values are 2-sided and were declared significant at the 5% level. All computations were carried out using commercially available software (SAS 9.0; SAS Institute).

**Results**

**Human breast cancer cell responses to TGFβ**

TGFβ regulation of ATM kinase activity and downstream phosphorylation targets does not depend on cycling status in nonmalignant MCF10A cells (19), but these cells are TGFβ responsive, whereas most breast cancers proliferate in the presence of active TGFβ signaling (30). Thus, we first established the relative TGFβ growth inhibition of MCF7, MDA-MB-231, Hs578T, and T47D breast cancer cell lines in comparison with MCF10A as a positive control (Fig. 1A). Among the breast cancer cell lines, only Hs578T cells were growth inhibited by TGFβ, similar to that observed in nonmalignant MCF10A cells. MCF7 and MDA-MB-231 cells were refractory to TGFβ-mediated growth regulation while T47D cells were slightly stimulated by addition of TGFβ.

![Graph](image-url)

**Figure 1.** TGFβ growth regulation and signaling in breast epithelial cell lines. A, the bar graphs show the growth response at 24 hours or 48 hours of TGFβ treatment in 5 breast epithelial cell lines. The untreated controls are designated as "UT" and "TGFβ" refers to TGFβ-treated cells. MCF10A cells show 14% (P = 0.38) and 35% (P = 0.02) growth inhibition at 24 hours and 48 hours of TGFβ treatment. Hs578T were growth inhibited by 9% (P = 0.36) at 24 hours and 22% (P = 0.004) at 48 hours of TGFβ treatment. Proliferation of MDA-MB-231 and MCF7 cells was unaffected by TGFβ whereas proliferation of T47D cells increased slightly. B, immunoblots of phospho-Smad2, total Smad2/3 and Actin from MCF7, T47D, Hs578T, MCF10A, and MDA-MB-231 cells treated with LY364947 for 48 hours followed by TGFβ for 30 minutes or sham treated. TGFβ treatment induced phosphorylation of Smad2, which was blocked by LY364947 pretreatment. These data indicate that TGFβ signaling through the type I receptor kinase is functional and LY364947 is effective in blocking the canonical pathway of TGFβ through TGFβRI. Quantifications of the ratios of phosphorylated protein/total protein normalized to untreated control are indicated below each lane.
Because breast cancer cell lines can selectively evade TGFβ growth regulation while maintaining signaling (26), we next examined activation of TGFβ canonical pathway as evidenced by Smad2 phosphorylation. Extracts from cells treated for 30 minutes with TGFβ showed a 2 to 12-fold increase in Smad2 phosphorylation, which was inhibited by pretreatment with LY364947, inhibitor of TGFβ receptor I (Fig. 1B). Thus all breast cancer cell lines, irrespective of their sensitivity to TGFβ-mediated growth inhibition, showed type I receptor kinase response to TGFβ treatment.

**Inhibition of TGFβ signaling increases radiosensitivity**

We then determined clonogenic survival as a measure of the radiation sensitivity of these human breast cancer cell lines and evaluated the effect of TGFβ signaling inhibition prior to irradiation. As previously reported (19), the radiation sensitivity of the TGFβ responsive, nonmalignant cell line MCF10A was significantly increased when subjected to inhibition of TGFβ signaling by TGFβ type I receptor kinase small molecule inhibitor LY364947 (Fig. 2). Moreover, the radiation sensitivity of TGFβ refractory cancer cell lines, MCF7 and T47D, and TGFβ growth sensitive cancer cell line, Hs578T, was increased following TGFβ inhibition (Fig. 2). The dose enhancement ratios at 10% survival (Table 1) varied between cell lines. Although representing a small sample of breast cancer cell lines, we found no apparent association between cancer cell subtype, estrogen receptor positivity or p53 mutation status, TGFβ growth sensitivity, and the effect of LY364947 pretreatment on radiosensitization.

**Inhibition of TGFβ signaling attenuates DDR in MDA-MB-231 cells in vitro and in vivo**

The MDA-MB-231 breast cancer cell line is designated triple negative and characterized as a basal subtype (31, 32). As in the other 3 breast cancer cell lines, LY364947 treatment prior to irradiation strongly radiosensitized MDA-MB-231 cells (Fig. 3A). Prior studies showed that compromised DDR exhibited by Tgfb1 null mouse epithelial cells or following inhibition of TGFβ signaling in nonmalignant human cells is due to significantly reduced ATM kinase activity (17, 19). ATM directly phosphorylates p53 at serine 15, and indirectly phosphorylates p53 at serine 20 (33). Consistent with increased radiosensitivity, p53 phosphorylation at both serine 15 and 20 was reduced

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**Table 1. Characteristics of breast cancer cell lines and radiation sensitization**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Characteristics</th>
<th>TGFβ-mediated growth inhibition at 48 h</th>
<th>Dose enhancement ratio at 10% survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF10A</td>
<td>Basal B</td>
<td>Neg                  WT</td>
<td>35%</td>
</tr>
<tr>
<td>Hs578T</td>
<td>Basal B</td>
<td>Neg                  MT</td>
<td>35%</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>Basal B</td>
<td>Neg                  MT</td>
<td>None</td>
</tr>
<tr>
<td>MCF7</td>
<td>Luminal</td>
<td>Pos                  WT</td>
<td>None</td>
</tr>
<tr>
<td>T47D</td>
<td>Luminal</td>
<td>Pos                  WT</td>
<td>None</td>
</tr>
</tbody>
</table>

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*ER: Estrogen receptor status.
**WT: Wild type; MT: Mutant.
***DER was estimated based on extrapolation to 10% survival.
in irradiated MDA-MB-231 cells treated with inhibitor (Fig. 3B). ATM also phosphorylates histone H2AX at serine 139, which is called γH2AX, detected as discrete foci at surrounding DNA double-strand breaks caused by ionizing radiation (34). The formation of γH2AX foci is widely used to monitor radiation-induced DNA breaks and to assay DNA rejoining defects (35). Consistent with increased radiosensitivity, p53 phosphorylation at both serine 15 and 20 was reduced in irradiated MDA-MB-231 cells (Fig. 3B). As found in our prior studies, radiation-induced γH2AX foci formation was markedly reduced by pretreatment with LY364947 (Fig. 3C).

To determine whether TGFβ inhibition could similarly affect the DDR in vivo, MDA-MB-231 cells were injected in immunocompromised mice to establish xenograft tumors. LY2109761 is a small molecule inhibitor that has similar activity as LY364947 (36), but is more pharmacokinetically stable, is orally available, and is the most potent in vivo inhibitor from this family of TGFβ type I receptor inhibitors (37). Mice-bearing MDA-MB-231 tumors were treated with LY2109761 for 2 hours before exposure to 3 Gy. As found in vitro, radiation-induced γH2AX foci were decreased in irradiated tumors treated with TGFβ inhibitor, LY2109761 compared with vehicle-treated tumors (Fig. 3D). Smad2 phosphorylation was slightly increased by radiation and both constitutive and radiation-induced Smad2 phosphorylation was decreased by LY2109761 (data not shown).

**TGFβ neutralization in combination with radiation increase tumor growth delay**

There are 3 pharmacologic routes to blocking TGFβ; neutralizing the ligand, inhibiting expression, or blocking
signaling (36). The pharmacokinetic properties of antibody and small molecule kinase inhibitors result in considerable differences in the duration of TGFβ signal modulation. At this point, the half-life of the TGFβ type I receptor kinase small molecule inhibitors may not be optimal for use in the context of fractionated radiotherapy. Alternatively, several TGFβ neutralizing antibodies are in clinical development that have shown safety and efficacy in fibrotic disorders (36, 38). We compared the efficacy of TGFβ ligand capture using 1D11 pan-TGFβ neutralizing antibodies to LY364947 in vitro using murine 4T1 breast tumor cells. Radiosensitization following 1D11 pretreatment (Fig. 4C) was comparable with that following LY364947 pretreatment (Fig. 4A), which indicated that TGFβ ligand sequestration and inhibition of the type I receptor kinase are functionally similar. As expected, both neutralizing antibody and small molecule inhibited Smad 2 phosphorylation activation in response to TGFβ.
Moreover, γH2AX foci were significantly reduced in irradiated tumors from mice treated with 1D11 antibodies compared with those treated with 13C4 control antibodies (Fig. 4E). TGFβ is a key contributor to the immunosuppressive tumor environment (39); radiation-induced TGFβ may hinder the development of anti-tumor immune responses that can be elicited by radiotherapy (40). Thus, to determine the effect of 1D11 antibody in conjunction with radiation treatment, we used 4T1 to establish preclinical breast tumors in immunocompetent mice. Mice-bearing 4T1 subcutaneous tumors were treated with TGFβ neutralizing antibody, 1D11, or a control antibody, 13C4, at 5 mg/kg, i.p. Tumors were sham-irradiated or irradiated with a 8 Gy single dose 24 hours later. Some tumors were randomly selected for harvest 1 hour after irradiation to assess γH2AX foci. As found with MDA-MB-231 cells treated with the small molecule inhibitor LY2109761, γH2AX foci were reduced in irradiated tumor treated by pan-specific antibody against TGFβ in vivo (Fig. 5A).

Although 4T1 tumor growth can be inhibited by TGFβ blockade when treated 1 day posttumor inoculation due to an effect on tumor-promoting immune cells (41), the growth rate of established tumors was not affected by TGFβ neutralizing antibody administered at this dose. As expected, irradiated tumors showed a significant tumor growth delay compared with unirradiated tumors (Fig. 5B and C). Tumor growth delay was further increased in mice that were both irradiated and treated with 1D11 neutralizing antibody compared with those that received radiation and 13C4 control antibody (Fig. 5D), with a significantly smaller average tumor volume (P < 0.05). No difference was observed with 1D11 treatment alone compared with no treatment controls (P = 0.77).

We speculated that increasing TGFβ antibody (50 mg/kg) in the context of multiple radiation fractions (3 × 12 Gy) could possibly provide greater benefit (Fig. 6A). Even at this high dose, a single administration of 1D11 alone in established tumors did not significantly reduce 4T1 tumor growth (P = 0.16). Tumor growth rate was profoundly
inhibited by treatment with multiple radiation fractions and further reduced by addition of 1D11 (Fig. 6B) compared with 13C4 (P < 0.05). Tumor weight at experiment termination (day 30) was also significantly reduced (P < 0.05) by combination treatment (Fig. 6C). No detectable toxicity, as evidenced by mouse husbandry or weight, was noted in mice treated with 1D11 antibodies for either dose (5 or 50 mg/kg) alone, or in combination with localized tumor irradiation (data not shown).

Discussion

We report herein that TGFβ inhibition increased radiation sensitivity measured by clonogenic assay of breast cancer cells in vitro and tumor growth delay in vivo. As previously shown for chemotherapy (24), inhibiting TGFβ promotes tumor response to radiation therapy. Our prior research using mouse and human epithelial cells showed that TGFβ inhibition compromises radiation-induced ATM kinase activity and downstream effectors of the DDR, resulting in increased cell killing measured by clonogenic assay (19). All 4 human breast cancer cells and a murine tumor cell line were radiosensitized independent of sensitivity to TGFβ-mediated growth inhibition, suggesting that this strategy would be effective across breast cancer subtypes.

Consistent with our earlier studies indicating diminished DDR following radiation exposure, TGFβ inhibition prior to irradiation also resulted in reduced phosphorylation of H2AX and p53 in cultured triple-negative MDA-MB-231 breast cancer cells. Either human MDA-MB-231 xenografts or murine 4T1 tumors in mice treated with TGFβ inhibitors prior to radiation exposure in vivo exhibited less γH2AX foci formation, a nuclear marker of the rapid molecular
radiation response. Moreover, 1D11 neutralizing antibodies enhanced the tumor growth delay after a single radiation exposure, which is consistent with a direct effect on radiosensitivity due to compromised DNA damage recognition. TGFβ inhibition before radiation treatment was most effective in combination with fractionated radiation therapy. Notably, 1D11 antibody did not by itself affect tumor growth rate when administered to established tumors. It is quite conceivable that effects on angiogenesis (37) and immunity (42) could also contribute to greater efficacy of TGFβ inhibition in combination with a fractionated course of radiation.

Teicher and colleagues showed that tumors secreting high levels of TGFβ are more resistant to chemotherapies such as cis-platinum (43, 44). Cis-platinum treatment of MDA-MB-231 breast cancer cells increased both TGFβ mRNA levels and the secretion of active TGFβ, leading to growth arrest and repair of damage: as a result cells became more resistant to cis-platinum killing (43). Anti-TGFβ antibodies enhanced cis-platinum induced DNA fragmentation in MDA-MB-231 cells, restoring cellular sensitivity to cis-platinum (44). Similarly, treatment of animals bearing cis-platinum–resistant tumors with TGFβ neutralizing antibody or with the TGFβ inhibitor, decorin, restores drug sensitivity of the tumor (43, 45, 46). The molecular mechanism(s) underlying this phenomenon remain to be elucidated; in our studies, TGFβ expression and activation lasts a period ranging from weeks to months in irradiated normal tissue (8), the concept of enhanced therapeutic index of radiotherapy from TGFβ inhibition is further supported by our in vitro and in vivo studies. Of particular interest is that either abrogation of TGFβ signaling by type I receptor kinase small molecule inhibitors or by ligand capture using neutralizing antibodies increased the radiosensitivity of diverse cancer cell lines in vitro and impaired DDR in vivo, which is the objective in the development of other therapies targeted to the DDR pathway (19, 20). Both MDA-MB-231 and 4T1 are models of triple-negative breast cancer, which has a poor prognosis. Recent studies from Reiss and colleagues show that TGFβ inhibition alone effectively reduces metastasis in a similar setting (37), which lends additional credence to the use of TGFβ inhibition in cancer therapy despite its tumor-suppressor activity. Our demonstration that short-term TGFβ neutralizing antibody increased tumor growth delay after a single radiation exposure and compromised tumor growth rate after fractionated radiation treatment in a syngeneic preclinical model without evidence of associated toxicity provides an additional route to therapeutic benefit. Our data argue for harnessing TGFβ inhibitors at the time of radiotherapy could translate protumor TGFβ biology into clinical benefit.

Disclosure of Potential Conflicts of Interest

M.H. Barcellos-Hoff has an unlicensed patent on the use of TGFβ inhibitors in radiotherapy. The other authors disclosed no potential conflicts of interest.

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References


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