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Advances in Brief

Transforming Growth Factor-β1 Mediates Cellular Response to DNA Damage *in Situ*[1]

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Abstract

Transforming growth factor (TGF)-β1 is rapidly activated after ionizing radiation, but its specific role in cellular responses to DNA damage is not known. Here we use *Tgfβ1* knockout mice to show that radiation-induced apoptotic response is TGF-β1 dependent in the mammary epithelium, and that both apoptosis and inhibition of proliferation in response to DNA damage decrease as a function of *TGF-β1* gene dose in embryonic epithelial tissues. Because apoptosis in these tissues has been shown previously to be p53 dependent, we then examined p53 protein activation. TGF-β1 depletion, by either gene knockout or by using TGF-β neutralizing antibodies, resulted in decreased p53 Ser-18 phosphorylation in irradiated mammary gland. These data indicate that TGF-β1 is essential for rapid p53-mediated cellular responses that mediate cell fate decisions *in situ*.

Introduction

TGF[3]-β1 orchestrates the response of multiple cell types to injury via its broad regulation of proliferation, apoptosis, and extracellular matrix composition (1). *Tgfβ1* gene deletion in mice demonstrates that TGF-β1 suppresses proliferation in a wide variety of epithelial cells and acts via true haploid insufficiency in its ability to protect against tumorigenesis (2). Resistance to TGF-β growth inhibition is a common feature of human breast, ovarian, and gastrointestinal cancer cells. Genetic mutations leading to loss of TGF-β signaling predispose certain tissues to develop cancer (3). The biological activity of TGF-β is constrained by its production as a latent complex consisting of TGF-β1 noncovalently associated with its processed NH_2-terminal pro-segment, called the LAP. Extracellular release from LAP, which is called activation, is a prerequisite for TGF-β1 to bind to its cell surface receptors. We discovered that TGF-β1 is rapidly activated in mouse mammary gland after IR (4). IR is one of a few exogenous agents known to cause latent TGF-β1 activation *in situ* (4, 5). The pleiotropic actions of TGF-β are well-suited to orchestrate cellular radiation responses to reestablish homeostasis. In particular, because TGF-β is a potent regulator of epithelial proliferation and apoptosis, we asked whether the IR-induced activation of TGF-β1 contributes to the cell fate decisions in response to DNA damage. To do so, we evaluated chronic depletion in *Tgfβ1* knockout mice, in which protein levels of TGF-β1 are chronically depressed by >90% in adult heterozygotes (6, 7), and transient depletion by administering TGF-β neutralizing antibodies. Together with our previous observations (4, 5), the data reported herein indicate that TGF-β1 signaling, initiated via the extracellular activation of latent TGF-β1, is essential for cellular responses to DNA damage.

Materials and Methods

Animal Studies. Adult 129Sv/C57BL/6 *Tgfβ1*, provided as breeding pairs by Dr. Anita Roberts (National Cancer Institute, NIH, Bethesda, MD), were bred at Lawrence Berkeley National Laboratory. Genotyping was performed by PCR as described (2). DNA was extracted using the GenomicPrep cell and tissue DNA isolation kit (Amersham Pharmacia, Piscataway, NJ) according to the manufacturer's instructions. HotStarTaq (Qiagen, Valencia, CA) DNA polymerase was used, and oligonucleotides were custom made by Operon (Alameda, CA). Pregnant NIH/OlaHsd *Tgfβ1* +/− dams were bred at University of California, San Francisco, and irradiated on day 12.5 of gestation, timed from observation of the vaginal plug. Embryos were dissected from the uterus 6 h after irradiation. Placental and tail tissue was digested for genotyping with Proteinase K. To transiently deplete TGF-β, adult BALB/c mice (B&K, Fremont, CA) were injected i.p. 3 h before irradiation with 0.5 μg of pan-specific TGF-β1 neutralizing antibody 2G7 purified IgG2b (8) or irrelevant immunoglobulin-matched antibody (Sigma Pharmaceuticals, St. Louis, MO). Unanesthetized adult mice were irradiated whole body with ^{60}Co γ-irradiation using a dose rate of 24 cGy/min to total dose of 5 Gy. Dosimetry was determined using a Victoreen ionization chamber before each experiment. Estrus was staged using cytological characteristics of vaginal smears at the time of irradiation and confirmed postmortem by uterine wet weight. Animals from each group were euthanized by CO_2 inhalation and cervical dislocation at the indicated times with institutional review and approval in accordance with American Association of Laboratory Animal Care guidelines.

Immunofluorescence. Freshly dissected inguinal mammary glands and embryos were embedded in Tissue-Tek compound (Sakura Finetek U.S.A., Inc., Torrance, CA), immediately frozen in a dry ice/ethanol bath, and stored at −80°C. Cryosections (5 μm) were cut at −30°C onto gelatin-coated coverslips. Immunostaining to differentiate between active and latent TGF-β1 was conducted as described previously (5). Sections were fixed using 2% buffered paraformaldehyde, followed by a 0.1 M glycine/PBS wash for the following antibodies: goat anti-LAP (R&D Systems, Minneapolis, MN), chicken anti-TGF-β1 antibodies (AF-101-NA, Lots # FS03 and # FS08; R&D Systems), Smad 2/3 (FL-425; Santa Cruz Biotechnology, Inc.), and PCNA fluorescein-conjugated monoclonal antibodies (DAKO, Carpinteria, CA). For PCNA, paraformaldehyde was followed by 10 min in methanol at 4°C. Phospho-specific antibodies to p53 Ser-18P (Cell Signaling Technologies, Beverly, MA) were used with sections fixed with 80% methanol for 10 min at −20C°, followed by 3-min fixation with 2% paraformaldehyde and quenching with 0.1 M glycine in PBS. After fixation, nonspecific sites were blocked before addition of primary antibodies were incubated with sections for 1 h at room temperature (p53 Ser-18P) or overnight at 4°C (LAP, TGF-β1, and Smad 2/3) in a humidified chamber. Sections were washed in PBS containing 0.1% BSA, before incubating with appropriate fluorochrome-conjugated secondary antibodies for 1 h in a dark humidified chamber and washed. Nuclei were counterstained with DAPI (Sigma) and mounted in Vectashield (Vector Laboratories, Burlingame, CA).

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[3] The abbreviations used are: TGF, transforming growth factor; LAP, latency-associated peptide; IR, ionizing radiation; Ser-18P, p53 serine 18 phosphorylation; PCNA, proliferating cell nuclear antigen; DAPI, 4′,6-diamidino-2-phenylindole; PALA, *N*-(phosphonacetyl)-L-aspartic acid.

Apoptotic Index. ApopTag (Intergen, Purchase, NY) was used by following the manufacturer's protocol with minor modifications. Briefly, fresh frozen sections were fixed in 1% paraformaldehyde, then in a precooled 2:1 ethanol: acetic acid mixture. Sections were blocked with the supernatant of 0.5% casein in PBS. The terminal deoxynucleotidyltransferase stock solution was used at a working strength of 30% for 1 h at 37°C. The stop reaction and FITC anti-digoxigenin antibody steps were followed as written. Sections were counterstained with DAPI and mounted with Vectashield.

Image Acquisition and Analysis. Images were obtained using a ×40, 0.75 numerical aperture Zeiss Neofluar objective on a Zeiss Axiovert equipped with epifluorescence. A multiband pass dichroic mirror, barrier filter, and differential wavelength filter wheel combination was used to selectively excite fluorochromes in sequence. Images were captured using a scientific grade, 12-bit charged coupled device (KAF-1400, 1317 × 1035, 6.8 μm square pixels) digital camera (Xillix, Vancouver, British Columbia, Canada). Images obtained from sections stained in parallel were captured with identical parameters and scaled using Scilimage (TNO Institute of Applied Physics, Delft, the Netherlands). False color images were compiled from gray-scale images of each fluorochrome.

The frequency of cells labeled with markers of apoptosis or proliferation were counted in at least four fields in duplicate sections from three mice or embryos. The number of epithelial nuclei in mammary epithelium or epidermis was counted in each image. Nuclear counts in embryonic liver were based on the total area of DAPI-stained nuclei divided by the mean area of 10 individually segmented nuclei. The percentage of positive cells was the marked/total × 100. Statistical significance of differences between genotypes was determined using the unpaired Student's *t* test (GraphPad PRISM).

Protein Extraction and Immunoblotting. Tissue extracts from the third and fifth mammary glands flash-frozen in liquid nitrogen were prepared, separated using reducing SDS-PAGE, and immunoblotted, and proteins of interest were detected using antibodies localized on film with chemiluminescence using a Pierce SuperSignal system (Pierce, Rockford, IL). Antibodies CM1, CM5, or Pab122 were used to determine total p53, and blots were probed for β-actin to assess loading. Exposed films were scanned and subjected to densitometric analysis.

Results

TGF-β1 Activation and Activity Are Reduced in Mammary Glands of Irradiated *Tgfβ1* +/− Mice. We have confirmed that TGF-β1 protein levels of *Tgfβ1* +/− adult mammary gland are reduced by >90% compared with wild type (7). To determine whether IR-induced TGFβ1 activation was also compromised, we localized active TGF-β1 by immunostaining. Active TGF-β1 was greatly reduced in irradiated *Tgfβ1* +/− mammary gland compared with wild-type tissue (Fig. 1*A*). To confirm that depletion of TGF-β1 resulted in decreased TGF-β1 signaling, we examined the induction of Smad 2/3 nuclear translocation. A marked induction of nuclear Smad 2/3 immunostaining was observed 1 h in irradiated *versus* sham-irradiated wild-type mice (Fig. 1*B*). The frequency of positively stained cells and the intensity of staining was reduced in irradiated *Tgfβ1* +/− mammary epithelium, indicating that *Tgfβ1* +/− mice are an appropriate model to study whether TGFβ1 depletion affects cell fate decisions.

Radiation-induced Apoptosis Is Absent in *Tgfβ1* +/− Mammary Epithelium. Previous studies demonstrated that a dose of 5 Gy IR induces a 2–3-fold increase in apoptosis that peaks at 6 h in mammary gland of nulliparous animals (9, 10). During studies of mammary development, we observed that the background frequency of apoptosis is related to the stage of estrus cycle, and that both proliferation and apoptosis peak at estrus (7). To ensure comparable background frequency, the animals were irradiated in estrus. The apoptotic index increased 3-fold in mammary glands of C57BL/6/129Sv *Tgfβ1* +/+ mice 6 h after whole-body exposure to a dose of 5 Gy of γ-radiation (Fig. 2*A*). In contrast, mammary epithelial apoptosis was not significantly increased after irradiation of *Tgfβ1*+/− mice and was in fact one-eighth the level of irradiated wild-type mice. Although physiological apoptosis in *Tgfβ1* +/− mammary epithelium at estrus is half that of wild-type mice, apoptosis is not generally depressed in *Tgfβ1* +/− mammary epithelium because levels are similar to wild-type at puberty and is increased during pregnancy (7). In addition, radiation-induced apoptosis in lymph node and spleen was similar in *Tgfβ1* +/− mice and wild-type mice (not shown). These data suggest that TGF-β1 affects cell fate decisions in response to DNA damage in a cell type-dependent manner.

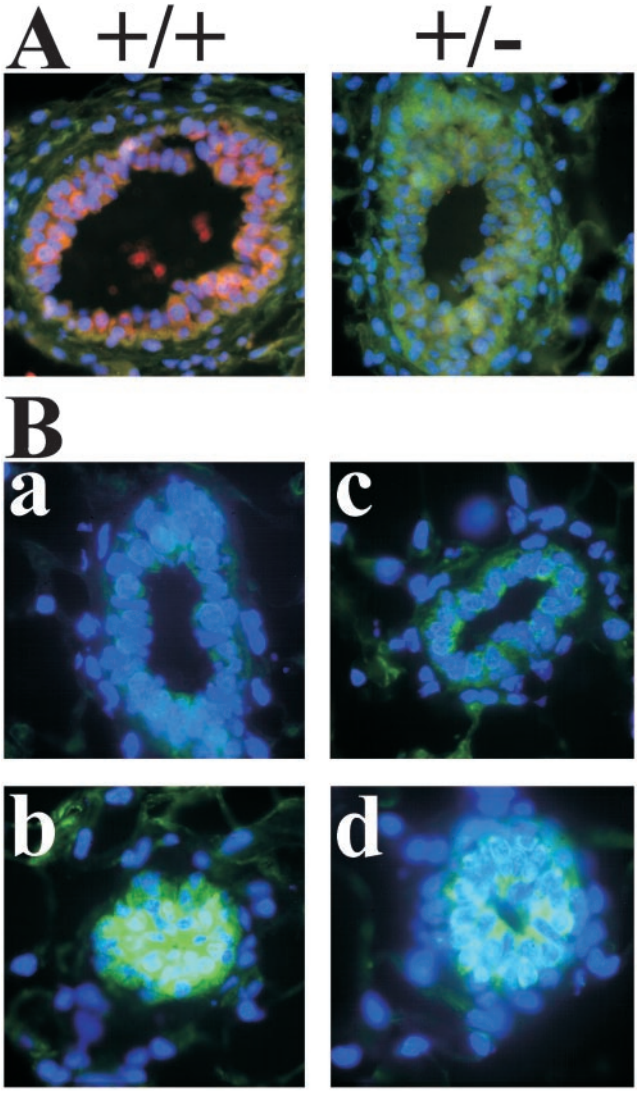

Fig. 1. Irradiated *TGF-β1* +/− mammary gland shows reduced levels of active TGF-β1 and Smad 2/3. *TGF-β1* +/− and +/+ mice were irradiated whole body to a dose of 5 Gy and killed 1 h later. *A*, false color digital micrographs of dual immunofluorescence of antigen-purified TGF-β1 antibody (*red*) and LAP antibody (*green*) visualized simultaneously with DAPI-stained nuclei (*blue*). Comparison of mammary gland tissue from irradiated *TGF-β1* +/+ and +/− mice indicates that TGF-β1 immunoreactivity (*yellow orange*) is greater in *TGF-β1* +/+ mice, whereas *TGF-β1*+/− mice show predominant LAP immunoreactivity (*green*). The prominent localization of TGF-β1 in the irradiated wild-type mice reflects radiation-induced activation (5). Note that all cells stain with antibodies to LAP before radiation exposure (7). *B*, false color digital micrographs of Smad 2/3 antibody (*green*) localized simultaneously with DAPI-stained nuclei (*blue*). Comparison of mammary gland cryosections from sham (*a* and *c*) or irradiated (*b* and *d*) *TGF-β1*+/+ (*a* and *b*) and +/− (*c* and *d*) mice indicates that IR induced significant Smad2/3 immunoreactivity. Immunofluorescence intensity was markedly reduced in irradiated *TGF-β1* +/− mice.

Absence of TGF-β1 in Embryonic Tissues Abrogates Apoptotic and Cell Cycle Inhibition in Response to IR. The radiation response of adult TGFβ1 null mice cannot be determined because *Tgfβ1* −/− genotype mice commonly die *in utero* (6). However, several embryonic tissues exhibit both a robust apoptotic response and cell cycle inhibition shortly after irradiation *in utero* (11). Therefore, 12.5-day pregnant *Tgfβ1* +/− dams were irradiated whole body with

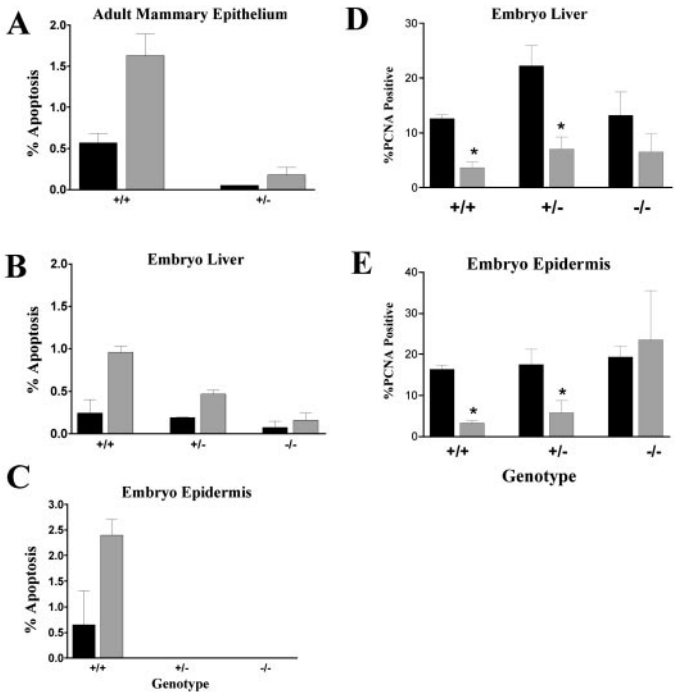

Fig. 2. *TGF-β1* gene dosage correlates with reduced apoptosis and cell cycle block in response to radiation. *A*, the frequency of apoptotic nuclei detected using terminal deoxynucleotidyltransferase-mediated nick end labeling reaction was determined in the mammary epithelium of *TGF-β1* +/− and +/+ mice (means; *bars*, SE; $n = 3$ animals). Sham-irradiated (■) and whole-body irradiated (▨) wild-type mice were significantly different (*t* test; $P = 0.02$). The irradiated *TGF-β1* heterozygote mice were not significantly different from sham-irradiated heterozygote mice but were significantly different from irradiated wild-type mice (*t* test; $P = 0.006$). Pregnant NIH/OlaHsd *TGF-β1* +/− dams were irradiated whole body (5 Gy) on day 12.5 of gestation. Embryos irradiated *in utero* were collected 6 h after irradiation. Apoptotic nuclei were detected using the terminal deoxynucleotidyltransferase-mediated nick end labeling reaction in liver **(*B*)** and epidermis **(*C*)** from *TGF-β1* +/+, +/−, and −/− embryos. Apoptosis was decreased in control *TGF-β1* +/− and −/− embryo tissues. Significantly increased apoptosis was absent from both liver and epidermis of irradiated *TGF-β1* +/− and −/− embryos. The frequency of cycling cells was detected using PCNA antibodies in sham-irradiated (■) and irradiated (▨) embryos. Radiation-induced cell cycle block was evidenced by a 2–3-fold reduction of PCNA-positive cells after irradiation *in utero* in the liver **(*D*)** and epidermis **(*E*)** from *TGF-β* +/+ and +/− embryos. The frequency of PCNA-positive cells was not significantly different between sham and IR embryos of −/− genotype, indicating abrogation of radiation-induced cell cycle block.

a dose of 5 Gy, and the embryos were collected 6 h later. Apotag-positive cells were counted in epidermis and liver (Fig. 2, *B* and *C*). Apoptosis increased 2–3-fold in epidermis and liver in irradiated wild-type embryos. Radiation-induced apoptosis was significantly decreased in *Tgfβ1* +/− embryos. *Tgfβ1* −/− embryos lacked an apoptotic response.

In rapidly proliferating tissues, IR can also induce a transient cell cycle block. Antibodies to PCNA were used to define the frequency of cells in cycle in embryonic tissues after IR (Fig. 2, *D* and *E*). Proliferation was reduced 2–3-fold after irradiation in liver and epidermis of both +/+ and +/− embryos. The frequency of proliferating cells was unaffected in irradiated −/− embryos. Together, these data demonstrate that TGF-β1 abundance dictates cell fate decision in irradiated embryonic as well as adult epithelial tissues.

p53 Stress Response Is Activated in Irradiated Mammary Gland. Apoptosis is p53 dependent in irradiated mammary gland and embryos (9–11). However, a recent report suggested that mammary gland lacks a classic p53 IR induction, as measured by nuclear immunoreactivity using the CM5 antibody (10). Because this antibody many be insensitive to p53 activation status determined by protein modifications, in the current study we used a phosphorylation state-specific antibody. Phosphorylation of Ser-18 (Ser-15 in human) is strongly associated with the cellular response to radiation damage (*e.g.*, apoptosis and cell cycle block) and contributes to p53 protein stability (12). The phosphorylation of Ser-18 promotes dissociation of p53 from the MDM2 protein, which otherwise directs p53 proteolysis. Immunoblotting of total mammary gland protein extracts showed that Ser-18P was undetectable in extracts from sham-irradiated tissue. Within 1 h of IR exposure, Ser-18P was significantly elevated and remained detectable up to 24 h after IR (Fig. 3*A*). Total p53 levels, detected using antibodies PAb122 or CM1, which are insensitive to phosphorylation status, were increased at 24 h after IR but unchanged during the period from 1 to 15 h (data not shown).

Because the mammary gland is composed of many cell types, we used immunofluorescence to determine the cellular localization of p53 bearing Ser-18P (Fig. 3*B*). Mammary epithelium from sham-irradiated mice showed minimal nuclear signal. The immunoreactivity of phospho-specific p53 Ser-18P antibodies was restricted to the nucleus and was punctate in irradiated mammary epithelial nuclei. Epithelial nuclear p53 Ser-18P immunostaining was significantly increased within 1 h of radiation exposure and remained prominent up to 24 h after irradiation.

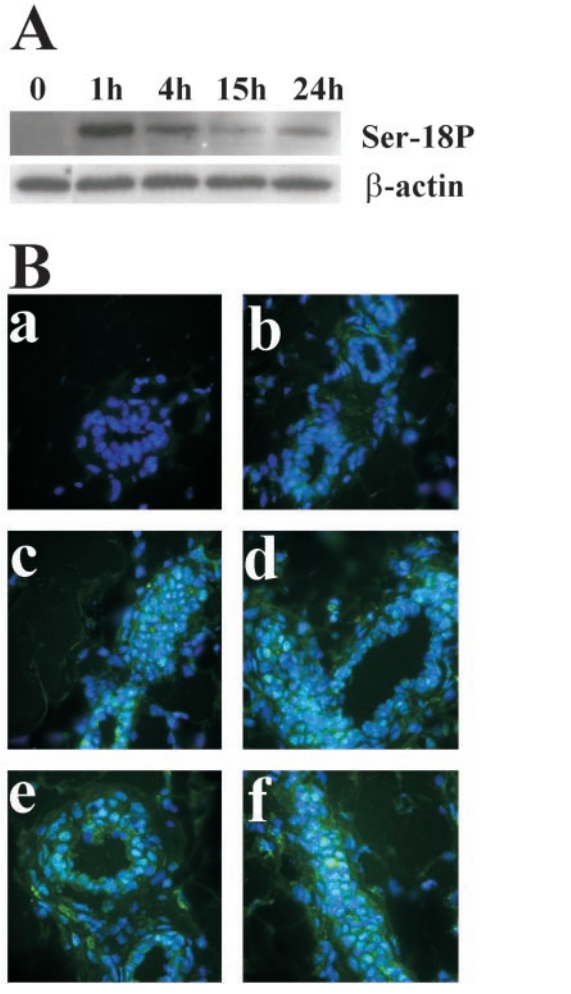

Fig. 3. p53 Ser-18P is induced in irradiated mammary epithelial cells. *A*, antibodies to Ser-18P p53 were used in Western blotting of total tissue protein extracts of irradiated BALB/c mammary tissue. No signal was evident in sham-irradiated tissue. A single band was detected at 1 h and was present up to 24 h after radiation exposure by Western blotting. *B*, false color images of immunofluorescence localization of p53 Ser-18 phosphorylation detected using secondary antibodies labeled with Alexa 488 (appears *green/turquoise*). Nuclei were counterstained with DAPI (*blue*). Immunofluorescence was absent from controls in which the primary antibody was deleted (*a*) and discernable in only a few epithelial cells in sham-irradiated tissue (*b*). Prominent nuclear immunoreactivity was evident throughout the epithelium from 1 h (*c*), 4 h (*d*), 15 h (*e*), and 24 h (*g*) after radiation exposure.

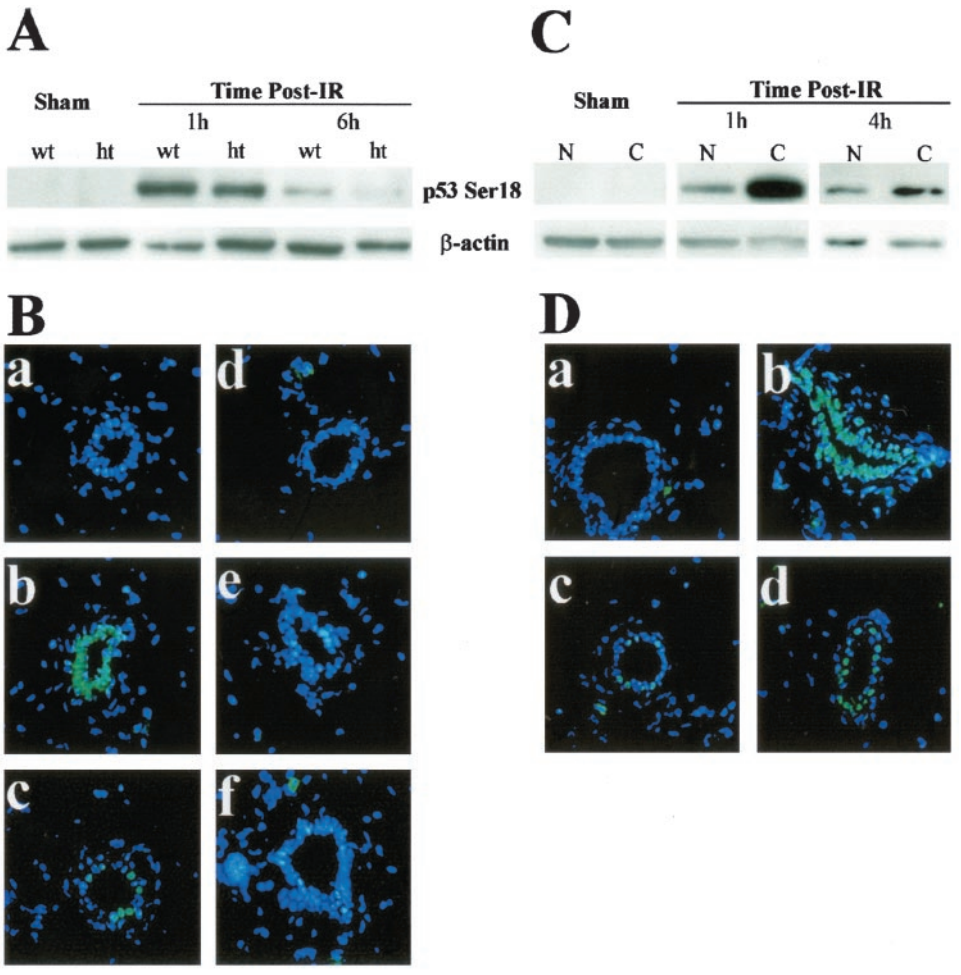

Fig. 4. Decreased radiation-induced p53 Ser-18 P after irradiation and chronic or transient TGF-β1 depletion. *A*, Western blot of tissue extracts from wild-type (*wt*) or *Tgfβ1* heterozygote (*ht*) mice sham and 1–6 h after IR. p53 Ser-18 phosphorylation was significantly reduced in *Tgfβ1* +/− mice 1–6 h after IR. *B*, p53 Ser-18P was localized as indicated in Fig. 3 using cryosections of C57BL/6/129Sv *TGF-β1* +/+ mice (*a–c*) or *TGF-β1* +/− mice (*d–f*) subjected to sham exposure (*a* and *d*) or irradiated with 5 Gy, 1 h (*b* and *e*) or 6 h (*c* and *f*) before sacrifice. Nuclear localization of p53 Ser-18P 1 h after IR was significantly reduced in *TGF-β1* +/− animals compared with wild-type animals. By 6 h, p53 Ser-18P was decreased in both genotypes. *C*, Western blot of tissue extracts from BALB/c adult female mice injected i.p. with an irrelevant IgG antibody as a control (*C*) or TGF-β1 neutralizing (*N*) antibody before irradiation. p53 Ser-18P was significantly reduced 1 h after IR when TGF-β1 neutralizing antibodies were administered before irradiation. *D*, nuclear immunolocalization of p53 Ser-18P was significantly reduced in animals treated with TGF-β neutralizing antibody. Mice received control (*a* and *b*) or TGF-β pan-isoform neutralizing monoclonal antibody (*c* and *d*) 3 h before sham exposure (*a* and *c*) or whole-body irradiation with 5 Gy (*b* and *d*). p53 Ser-18P was localized in cryosections as indicated in Fig. 3.

Chronic or Transient TGF-β1 Depletion Inhibits p53 Ser-18 Phosphorylation. Immunoblots of p53 Ser-18P using total protein extracts from wild-type mice showed a massive induction of p53 phosphorylation 1 h after IR (Fig. 4*A*). Ser-18P detection was decreased at least 4-fold in *Tgfb1* +/− compared with wild-type mice at both 1 and 6 h, although Ser-18P p53 levels decreased ~10-fold between 1 and 6 h in wild-type mice. Total p53 in wild-type and heterozygote mammary extracts measured by CM1 or CM5 were similar (data not shown), suggesting that phosphorylation, rather than abundance, was severely and persistently compromised. The immunolocalization of p53 Ser-18P in irradiated *Tgfβ1* +/− mammary epithelium was also decreased compared with that of wild-type mice (Fig. 4*B*). Nuclear p53 Ser-18P immunofluorescence was significantly reduced at 1 h after irradiation in the *Tgfβ1* heterozygote compared with wild-type mammary epithelium. The difference between irradiated wild-type and *Tgfβ1* +/− mice was less pronounced at 6 h after irradiation but was still attenuated in *Tgfβ1* +/− mammary epithelial cells.

Chronic depletion in *Tgfβ1* +/− mice could perturb aspects of cell physiology that modify the p53 radiation response. To test whether TGF-β1 directly affected the radiation response, pan-specific TGF-β1 neutralizing antibodies were administered i.p. 3 h before irradiation. Our previous studies had demonstrated the efficacy of this timing, route, and antibody dose in blocking TGF-β1-mediated extracellular matrix remodeling (5). Immunoblotting showed that p53 Ser-18P after radiation exposure was reduced from neutralizing antibody-treated mice compared with animals treated with control antibody (Fig. 4*D*). p53 Ser-18P was reduced 5-fold at 1 h after IR in animals receiving TGF-β neutralizing antibody compared with those receiving control antibody. This difference was less evident at 4 h after irradiation. Likewise, nuclear localization of p53 Ser-18P determined by immunofluorescence staining was significantly reduced 1 h after IR when TGF-β1 was transiently depleted before irradiation (Fig. 4*D*). As seen in the gene knockouts, TGF-β1 neutralizing antibody treatment did not alter levels of total p53, as detected using either CM1 or CM5 antibody (data not shown), indicating that TGF-β availability affected p53 posttranslational modification rather than total protein abundance.

Discussion

We tested the hypothesis that TGF-β1 modulates the type and degree of cellular damage responses *in situ*. The decision of a cell to undergo apoptosis in response to DNA damage is commonly attributed to the level of DNA damage and certain cellular competencies that are poorly understood *in vivo*. The data reported here reveal a surprising TGF-β dependence for cellular response to DNA damage. Upon finding that radiation-induced apoptosis was undetectable in *Tgfβ1* +/− mammary gland, we examined the apoptotic response in embryonic tissues as a function of *Tgfβ1* gene dosage. Radiation-induced apoptosis correlated with TGF-β abundance in both liver and epidermis. In addition, the IR-induced proliferative block was completely absent in irradiated *Tgfβ1* null embryo tissues. Because both responses have been shown to be p53 dependent (9–11), we then examined the phosphorylation of Ser-18 associated with rapid p53 activation (12). Depletion of TGF-β1 abrogated p53 phosphorylation in mammary glands in both the chronically depleted knockout mice or after transient inhibition using TGF-β1 neutralizing antibodies.

TGF-β is an important tumor suppressor (1, 3, 13). Likewise, the p53 response is an important mechanism of tumor suppression, as is underscored by the high frequency of cancer in Li-Fraumeni syndrome in which p53 malfunctions, by studies in *p53* knockout mice, and by the high frequency of mutant p53 found in human tumors and cancer cells (14). Activation of p53 in damaged cells may induce cell cycle progression delays expressed through either the production of G_1-S or G_2-M phase transition blocks that provide time for DNA repair (15). Alternatively, certain cells undergo p53-mediated apoptosis (16). The factors that influence which response occurs include the type of cell, the level of damage, and cell cycle status (17).

In response to DNA damage, most studies have focused on intracellular p53 to understand individual cells, whereas tissue processes are coordinated by TGF-β1. There are similarities between p53 and TGF-β1 that equip each to participate in damage control. Both are abundant in latent forms that restrict activity until certain protein modifications occur, which includes redox sensitivity (18, 19), that endow them with the capability of being rapidly activated. Rapid activation of the p53 stress response is predominantly posttranslational. Covalent protein modifications that affect p53 stability and activity include phosphorylation, dephosphorylation, acetylation, and deacetylation (20). These modifications can, in turn, affect the binding partners, localization, activity, and degradation of p53. The TGF-β latent complex is abundant in bound and circulating forms, and its biological activity is controlled by extracellular processing that releases TGF-β from LAP, which may be further modulated by binding to extracellular proteins. TGF-β activation acts as the switch to initiate tissue response to damage in physiological processes including inflammation, wounding, and angiogenesis. These common properties enable both p53 and TGF-β to perform rapidly in response to significant DNA damage.

We have shown that latent TGF-β1 activation can occur via free radical generation by radiation and other sources, thus endowing TGF-β1 with the ability to act as an extracellular sensor of oxidative stress (19). In addition to IR (4), other DNA-damaging agents induce TGF-β1 activation, including PALA (21), cisplatin (22), and alkylating agents (23). Studies using keratinocytes from *Tgfβ1* knockout mice also support a functional, rather than accessory, role for TGF-β in damage response. PALA-induced gene amplification was elevated >100 times in *Tgfβ1* null keratinocytes compared with wild-type cells, whereas addition of exogenous TGF-β1 to knockout cells reversed instability (21). Similar to our observations in irradiated *Tgfβ1* −/− embryos, *Tgfβ1* −/− keratinocytes lack the typical PALA-induced, p53-dependent G_1 arrest.

Our data suggest a previously unsuspected action of TGF-β as an extracellular mediator of intracellular responses to DNA damage. A number of studies have reported that p53 status can affect responses to TGF-β1 and *vice versa* (24). Both are induced by a variety of cytotoxic agents, specifically IR, and both undergo autoregulatory translational and transcriptional control that moderate later events. The rapid induction of Smad 2/3 immunoreactivity that we observed in irradiated mammary tissue and the observation that TGF-β1 enhances the stress response after UV irradiation (25) suggest that there may be a direct interaction between the TGF-β signaling and damage response pathways.

Altered responsiveness to TGF-β1 has been broadly implicated in breast cancer progression (3, 26). We and others have argued that conversion to TGF-β1 growth resistance is a critical juncture in the evolution of malignant behavior (27), which allows TGF-β to stimulate tumor progression at later stages of carcinogenesis (13). We have determined that TGF-β1 availability affects cellular and p53 responses in irradiated tissues. These data suggest that TGF-β1 should be considered as a key regulator of genomic integrity. Its early loss, by whatever means, could contribute to genome instability through reduced action of p53.

Acknowledgments

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