Inactivation of *gadd45a* Sensitizes Epithelial Cancer Cells to Ionizing Radiation *In vivo* Resulting in Prolonged Survival

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Abstract

Ionizing radiation (IR) therapy is one of the most commonly used treatments for cancer patients. The responses of tumor cells to IR are often tissue specific and depend on pathway aberrations present in the tumor. Identifying molecules and mechanisms that sensitize tumor cells to IR provides new potential therapeutic strategies for cancer treatment. In this study, we used two genetically engineered mouse carcinoma models, brain choroid plexus carcinoma (CPC) and prostate, to test the effect of inactivating *gadd45a*, a DNA damage response p53 target gene, on tumor responses to IR. We show that gadd45a deficiency significantly increases tumor cell death after radiation. Effect on survival was assessed in the CPC model and was extended in IR-treated mice with gadd45a deficiency compared with those expressing wild-type gadd45a. These studies show a significant effect of gadd45a inactivation in sensitizing tumor cells to IR, implicating gadd45a as a potential drug target in radiotherapy management. [Cancer Res 2008;68(10):3579–83]

Introduction

Ionizing Radiation (IR) is one of the most commonly used therapies in oncology. Tumor cell responses to IR are tissue specific and depend greatly on the pathway defects present within tumors. Therefore, understanding the molecular mechanisms of the cellular responses to IR is essential for managing and improving this mode of cancer treatment.

The tumor suppressor gene *Trp(p53)* is a key player in the cell response to stress signals, including IR. For example, following IR treatment, murine thymocytes undergo rapid p53-dependent apoptosis, fibroblasts enter irreversible p53-dependent cell cycle arrest, whereas epithelial cells usually go through reversible cell cycle arrest (1). Stress signals, including DNA damage and oncogenic events, induce p53 activity eliciting differential expression of p53 target genes. These downstream genes can be divided into major groups categorized by established p53 roles in a given biological response. The best characterized of these include cell cycle arrest genes [e.g., *p21(Cdkn1), gadd45a*, and *14-3-3σ*] and apoptosis genes [e.g., *bax, Apaf1, puma, p53AIP1*, and *noxa*; refs. 2, 3]. Among p53-regulated cell cycle control genes, *gadd45a* has been shown to play an important role in DNA damage–induced cell responses. For example, gadd45a deficiency causes defective UV-induced nucleotide excision repair (4). Gadd45a participates in the proper control of the G_2-M checkpoint in response to UV radiation and of the S-phase checkpoint under multiple conditions of nutrient deprivation (5–7). *Gadd45a*-null mouse embryonic fibroblast cells exhibit increased aneuploidy accompanied with abnormal centromere amplification; when exposed to IR, *gadd45a* knockout mice also show increased lymphomagenesis compared with control mice (8). Interestingly, *in vivo* studies have shown that gadd45a inactivation also causes abnormal p38 mitogen-activated protein kinase phosphorylation, T-cell hyperproliferation, and a lupus-like autoimmune disease in mice (9, 10). In addition to p53, BRCA1 and FOXO3a have also been shown to activate *gadd45a* gene expression (11, 12).

In addition to cell cycle control, there is evidence that gadd45a is also involved in DNA damage–induced apoptosis. For example, gadd45a prevents UV-induced skin tumors and promotes keratinocyte apoptosis in mice via the p38 and p53 pathways (13). Similarly, gadd45a suppresses Ras-induced mammary tumorigenesis by p38-mediated cell cycle arrest and apoptosis (14). Overexpression of gadd45a in HeLa cells induces apoptosis through translocation of Bim to mitochondria (15). However, little is known about the role of gadd45a in control of apoptosis in the cellular response to IR *in vivo*.

In the current study, we used in genetically engineered mouse models of spontaneous brain and prostate carcinoma to investigate the role of gadd45a role in epithelial tumor responses to IR treatment. We found that gadd45a inactivation increased the *in vivo* sensitivity of carcinoma cells to IR resulting in significantly delayed tumor progression.

Materials and Methods

Mice. The transgenic TgT_{121} brain tumor mouse model (16, 17), the $TgAPT_{121}$ prostate carcinoma mouse model (18), and mice harboring a homozygous deletion of the *gadd45a* gene (8) or of the *p21* gene (19) were previously described. TgT_{121};$gadd45a^{-/-}$ and TgT_{121};$gadd45a^{+/-}$ were generated by crossing hemizygous TgT_{121} mice with $gadd45a^{-/-}$ mice, and TgT_{121};$p21^{-/-}$ and TgT_{121};$p21^{+/-}$ were generated by crossing hemizygous TgT_{121} mice with $p21^{-/-}$ mice. $TgAPT_{121}$;$gadd45a^{-/-}$ mice were generated by crossing TgT_{121} mice with $gadd45a^{-/-}$ mice. To produce homozygous null backgrounds, transgenic mice that were heterozygous at the desired locus were crossed to respective homozygous null animals. In every case, the oncogenic transgene was maintained in the hemizygous state.

Radiation treatment. To assess brain tumor cell responses, 2-mo-old mice (male and female) were treated with one 10-Gy dose whole-body radiation and then euthanized 4.5 h after treatment for terminal deoxyribonucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay. A different group of mice were treated with the same dose of irradiation and were injected with bromodeoxyuridine (BrdUrd; 30 μg/g body weight) 4.5 h after treatment; the mice were euthanized 1 h after the injection and brain tissues were fixed for immunohistochemical assay. For analysis of prostate tumor cells, 2-mo-old male mice were treated with one

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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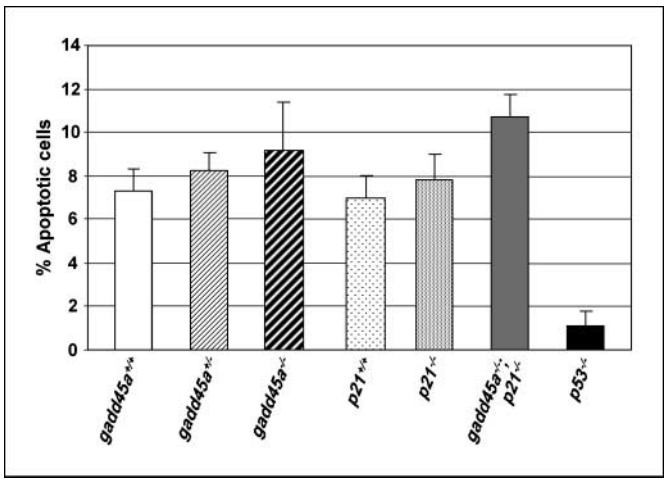

Figure 1. Gadd45a and p21 are not effectors of the p53-dependent apoptotic response to oncogenic stress. Brain sections from three or more mice of each genotype were analyzed by TUNEL. Average apoptotic indexes and SDs were calculated as described in Materials and Methods. Inactivation of either gadd45a or p21 does not significantly affect p53-dependent apoptosis; deficiency in both effectors causes a small but statistically significant increase of apoptosis, potentially due to relaxation of feedback regulation ($P < 0.05$).

10-Gy whole-body dose. For survival analysis, 2-mo-old TgT_{121};$gadd45a^{-/-}$ and TgT_{121};$gadd45a^{+/+}$ mice were irradiated (heads only) at a dose of 2 Gy/d for a total of 10 Gy with a 1-d interval after receiving treatment for 2 d. Mice were anesthetized with 2.5% Avertin (0.3 mL/20 g body weight) before irradiation. Mice were euthanized when signs of illness were present (e.g., domed head, lethargy).

TUNEL and proliferation assays. Brain and prostate tissues were fixed, embedded, and sectioned as described (20). Apoptotic cells were detected in sections by the TUNEL assay (17, 20). For each mouse, 8 to 10 different fields were counted under microscope. At least three mice of each genotype were analyzed, and the counts of apoptotic indexes were averaged and the SDs within each genotype group were calculated (represented by error bars). Proliferation rate of tumor cells was measured by BrdUrd immunostaining as previously described (20).

Statistics. *T* tests were used to evaluate the difference in apoptosis level between different groups of mice. Log-rank tests were used for survival analysis.

Results

We previously established a mouse brain epithelial [choroid plexus epithelium (CPE)] tumor model, TgT_{121}, in which choroid plexus carcinoma (CPC) development is initiated by cell-specific transgenic expression of T_{121}, an NH_2-terminal fragment of SV40 T antigen that inactivates pRb and related proteins, p107 and p130 (21). T_{121} acutely induces aberrant CPE cell proliferation accompanied by p53-mediated apoptosis and predisposes to aggressive tumor growth, which occurs on p53 inactivation. Tumors are histologically indistinguishable from human CPCs (17). To evaluate the contribution of *p53* downstream genes to p53 tumor suppression function in TgT_{121} mice, we generated TgT_{121};$gadd45a^{-/-}$ mice, and found that, unlike p53 deficiency, gadd45a deficiency does not affect the apoptosis level induced by pRb function loss (Fig. 1). To determine whether the response to irradiation was affected by gadd45adeficiency, we treated TgT_{121};$gadd45a^{-/-}$, TgT_{121};$gadd45a^{+/-}$, and TgT_{121};$gadd45a^{+/+}$ mice with a single dose of IR to the head (10 Gy) and examined acute

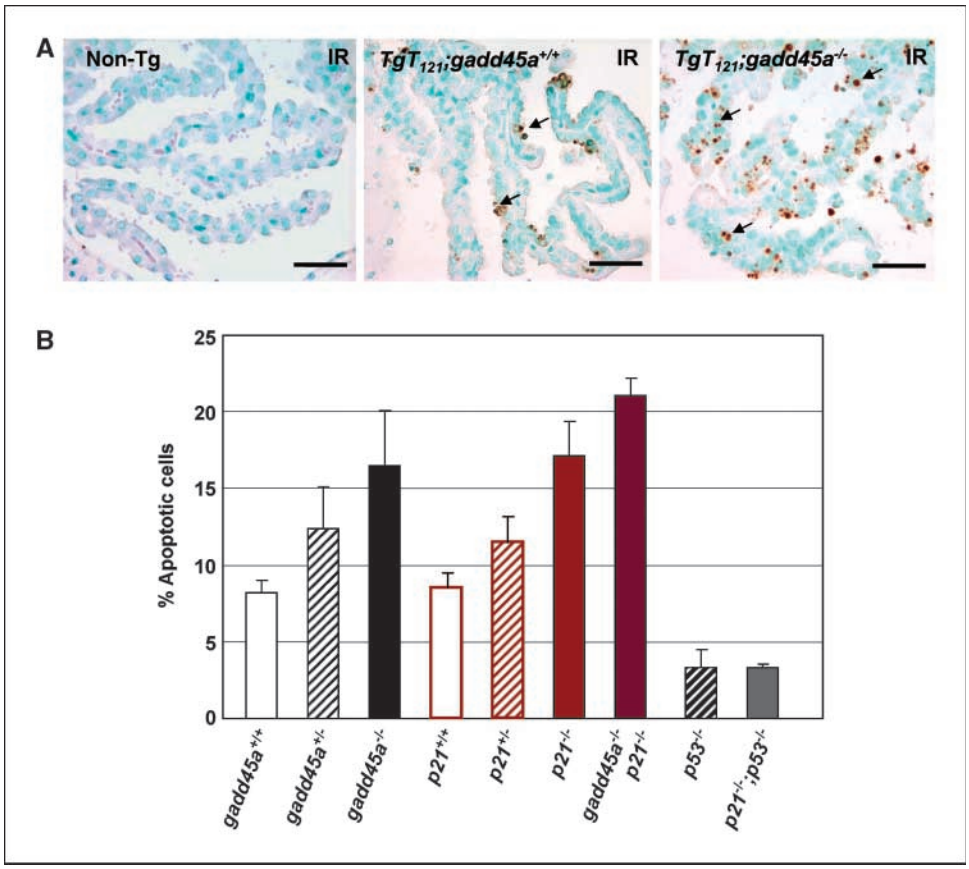

Figure 2. *Gadd45a* or *p21* deficiency sensitizes brain epithelial cancer cells to IR *in vivo*. *A,* apoptosis of CPE tumor cells after IR treatment. Mice, 6 to 8 wk old, were treated with one dose of 10-Gy radiation and euthanized 4.5 h after IR treatment. *Arrows,* representative apoptotic cells detected by TUNEL. TgT_{121};$gadd45a^{-/-}$ CPE contained significantly more apoptotic cells compared with TgT_{121};$gadd45a^{+/+}$ CPE. Nontransgenic (*Non-Tg*) CPE had a minimal level of apoptosis. *Bar,* 50 μm. *B,* quantitative analysis of apoptosis. All mice were TgT_{121} positive with indicated genotypes (three or more mice of each genotype were analyzed as described in Materials and Methods). *Gadd45a* or *p21* deficiency increased the sensitivity to IR-induced apoptosis. Loss of both *Gadd45a* and *p21* increased the effect but not additively. Increased IR-induced apoptosis was p53 dependent as evidenced by its reduction in p53-null backgrounds.

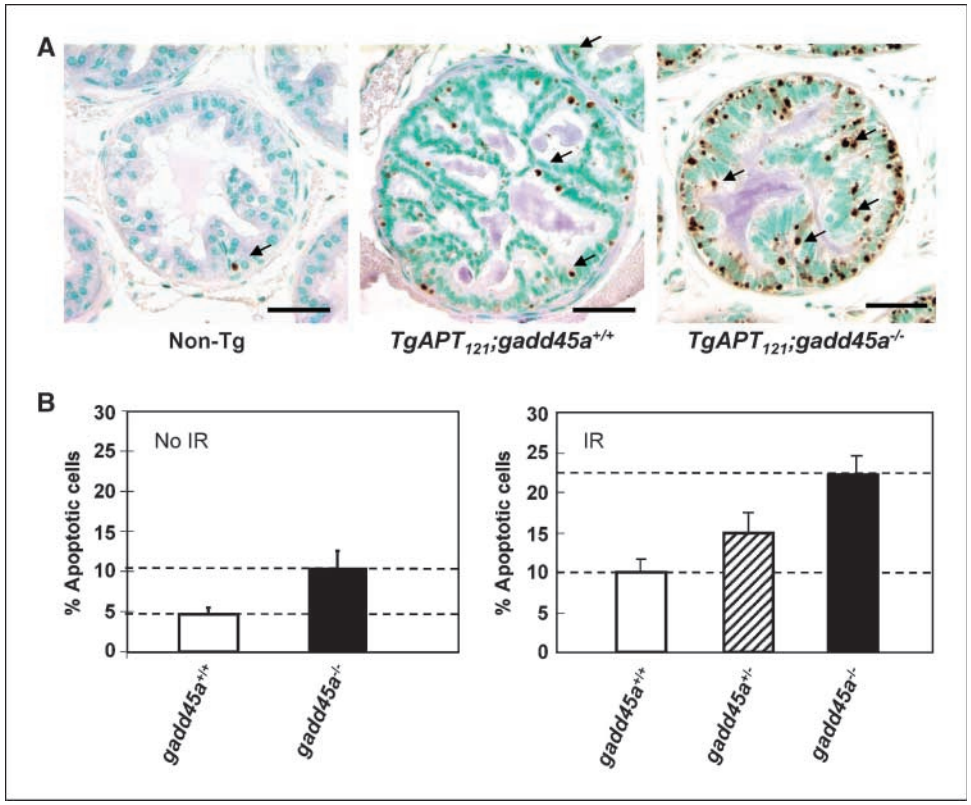

Figure 3. *Gadd45a* deficiency sensitizes prostate tumor cells to IR *in vivo*. *A,* apoptosis of mouse prostate cancer cells after IR. Male mice (2–3 mo old) were treated with a single dose of whole-body (10 Gy) radiation and euthanized 4.5 h after IR treatment. *Arrows,* representative apoptotic cells detected by TUNEL. *Gadd45a* deficiency increased the sensitivity of these cells to IR. *B,* quantitative assessment of data as described in Materials and Methods. *Gadd45a* deficiency caused an ~2-fold increase in apoptosis in the absence (*left*) or presence (*right*) of IR treatment.

effects within the tumor 4.5 hours after the treatment. Apoptosis, measured by the TUNEL assay, was significantly increased in TgT_{121};$gadd45a^{-/-}$ tumors (16.5 ± 3.6%; n = 5) compared with the TgT_{121};$gadd45a^{+/+}$ controls (8.2 ± 0.8%; n = 5; P < 0.05). TgT_{121};$gadd45a^{+/-}$ tumors yielded an intermediate apoptosis index (12.4 ± 2.7%; n = 4; Fig. 2). The CPE of nontransgenic mice, both $gadd45a^{+/+}$ and $gadd45a^{-/-}$, contained a very low level of IR-induced apoptosis(<1%; data not shown).

Another *p53* downstream cell cycle control gene, *p21*, also plays an important role in the cellular response to DNA damage signals, eliciting G_1 or G_2-M cell cycle arrest (19, 22, 23). Thus, we also examined the IR-induced apoptosis in CPE tumors of TgT_{121};$p21^{-/-}$ mice. Similar to that of TgT_{121};$gadd45a^{-/-}$ mice, without IR treatment the average CPE tumor cell apoptosis index of TgT_{121};$p21^{-/-}$ mice was about the same as that of TgT_{121};$p21^{+/+}$ mice. However, with IR treatment the average apoptosis index in tumors of TgT_{121};$p21^{-/-}$ mice (17.1 ± 2.3%; n = 3) was ~2-fold greater than that of TgT_{121};$p21^{+/+}$ mice (8.5 ± 0.8%; n = 5; P < 0.05), with an intermediate level of apoptosis in the tumors of TgT_{121};$p21^{+/-}$ mice (11.5 ± 1.7%; n = 4; Fig. 2). Inactivating both *gadd45a* and *p21* genes caused an even higher level of IR-induced apoptosis (21.1 ± 1.1%; n = 5) compared with inactivating either gadd45a or *p21* alone (Fig. 2). Although the apoptosis level was significantly increased, there was no significant change in the tumor cell proliferation rates in TgT_{121};$gadd45a^{-/-}$ and TgT_{121};$p21^{-/-}$ mice compared with TgT_{121} control mice as determined by BrdUrd incorporation (data not shown).

These data indicate that inactivation of *p53* downstream cell cycle arrest genes *gadd45a* or *p21* sensitizes epithelial tumor cells to DNA damage *in vivo*. To determine whether these effects were mediated by p53, we measured the IR-induced apoptosis levels of TgT_{121};$p53^{-/-}$ and TgT_{121};$p21^{-/-}$;$p53^{-/-}$ mice, which were 3.3% + 1.2% (n = 4) and 3.3% + 0.2% (n = 4), respectively, implying that the increased IR-induced cell death in TgT_{121};$p21^{-/-}$ mice, like the oncogene-induced death, was dependent on p53 function (Fig. 2).

To determine whether IR-induced tumor cell death enhancement by gadd45 or p21 deficiency was specific to CPE tumors, or might be more broadly applicable, we examined IR-induced apoptosis in a prostate cancer mouse model, $TgAPT_{121}$. In this model, tumors were initiated by prostate epithelial expression of T_{121} using the probasin promoter. Aberrant proliferation and abundant apoptosis occurs in prostate luminal epithelial cells, causing the development of mouse prostatic intraepithelial neoplasia and establishing the selective pressure for tumor progression. However, unlike the CPE model and a T_{121}-induced mammary gland tumor model (17, 24), the apoptosis is not mediated by p53 but rather by phosphatase and tensin homologue (18). $TgAPT_{121}$ male mice display slow progression to well-differentiated prostate adenocarcinoma (18). We generated $TgAPT_{121}$;$gadd45a^{+/+}$, $TgAPT_{121}$;$gadd45a^{+/-}$, and $TgAPT_{121}$;$gadd45a^{-/-}$ mice. Male mice at 2 to 3 months of age were treated with one dose of IR (10 Gy; whole body) and prostate apoptosis was measured by TUNEL. Nontransgenic prostate apoptosis was very low (<1%; Fig. 3*A*). $TgAPT_{121}$;$gadd45a^{+/+}$ prostate apoptosis increased to 10.0 ± 1.7% (n = 6; P < 0.05; Fig. 3*B*), whereas $TgAPT_{121}$;$gadd45a^{+/-}$ prostates showed intermediate levels of apoptosis (14.9 ± 2.6%). Once again, gadd45a deficiency caused a high level of apoptosis in response to IR (22.1 ± 2.4%; n = 6). Therefore, as in the brain epithelial tumor model, inactivating gadd45a sensitizes prostate cancer cells to IR *in vivo*. It is worth to note that in the absence of IR, gadd45 deficiency also caused increased apoptosis level without IR.

Because apoptosis levels are a critical factor in over all tumor growth rates and animal survival, we further examined the effect of

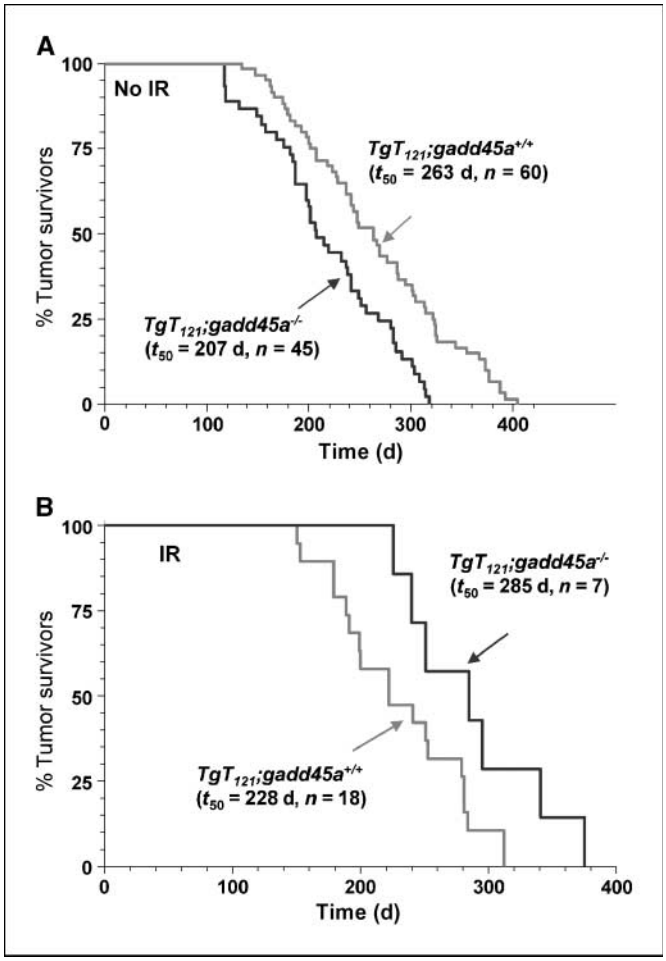

Figure 4. *Gadd45a* inactivation extended survival from brain tumors in IR-treated TgT_{121} mice. *A,* survival of TgT_{121};$gadd45a^{+/+}$ mice and TgT_{121};$gadd45a^{-/-}$ mice without IR treatment. Kaplan-Meier curves showed a shorter survival of TgT_{121};$gadd45a^{-/-}$ mice (t_{50} = 207 d; n = 45) compared with TgT_{121};$gadd45a^{+/+}$ mice (t_{50} = 263 d; n = 60). $P < 0.05$, log-rank test. *B,* after sublethal IR treatment to the head (see Materials and Methods), TgT_{121};$gadd45a^{-/-}$ mice had a better survival (t_{50} = 285 d; n = 7) than TgT_{121};$gadd45a^{+/+}$ mice (t_{50} = 223 d; n = 18; $P < 0.05$, log-rank test).

gadd45a inactivation on the survival of IR-treated mice. Brain carcinomas of TgT_{121} mice develop life-threatening tumors with consistent timing, whereas prostate adenocarcinomas of $TgAPT_{121}$ mice do not reproducibly affect survival (18, 25). Therefore, the brain tumor model was used for survival studies. In the absence of IR, TgT_{121};$gadd45a^{-/-}$ mice had a shorter survival time (t_{50} = 207 days; n = 45) than did TgT_{121};$gadd45a^{+/+}$ mice (t_{50} = 263 days; n = 60; Fig. 4*A*; $P < 0.05$), indicating a tumor suppression function of *gadd45a* gene. In the IR treatment study, 2-month-old TgT_{121};$gadd45a^{+/+}$ and TgT_{121};$gadd45a^{-/-}$ mice were irradiated by a ^{137}Cs irradiator using a modified clinical protocol. Consistent with an increased apoptosis index, TgT_{121};$gadd45a^{-/-}$ mice (t_{50} = 285 days; n = 7) lived significantly longer than TgT_{121};$gadd45a^{+/+}$ mice (t_{50} = 228 days; n = 18; $P < 0.05$; Fig. 4*B*). In addition, the survival time of IR-treated TgT_{121};$gadd45a^{+/+}$ mice was shortened by ~30% after IR treatment compared with untreated mice. Also noteworthy, inactivating both *gadd45a* and *p21* genes increased IR-induced apoptosis more than did inactivation of either gene alone (Fig. 2*B*). However, the effect of inactivating gadd45a or p21 was not additive, suggesting either that a maximum detectable level was reached or that there is overlap in IR-mediated DNA damage checkpoints. Interpretation of survival studies in mice with the compound deficiency (Supplementary Fig. S1) was confounded by the observation that all TgT_{121} mice with a p21 deficiency developed severe hydrocephalus independent of IR treatment.

Discussion

These studies show that *gadd45a* inactivation sensitizes both brain and prostate epithelial cancer cells to IR treatment. Tumor progression is slowed and survival extended in the brain carcinoma mouse model. Interestingly, a previous clinical report showed that *gadd45a* expression levels correlated with radiotherapy prognosis in a group of cervical cancer patients (26). Patients with relatively low *gadd45a* expression induction showed better prognosis following radiotherapy than patients with high *gadd45a* expression levels (26). Our data provide a possible explanation for this observation. Together, these data suggest that gadd45a may serve as a radiotherapy prognosis indicator and that inactivating *gadd45a*, possibly through small molecule inhibitors, could be used in conjunction with radiation to improve response to treatment.

Enhanced apoptotic response to IR in the absence of Gadd45a or p21 seems to depend on p53 function. Whereas CPC tumor cell apoptosis was increased after IR treatment in TgT_{121};$p21^{-/-}$ mice compared with TgT_{121};$p21^{+/+}$ mice, the effect was negated on further deficiency in p53 (Fig. 2*B*). Hence, this combined therapeutic approach is predicted to be effective only for tumors that retain p53 function. Interestingly, in the clinical study mentioned above, tumors of all patients included in the study were genotypically wild-type for p53 (26). In the brain tumor system, inactivation of p21 was associated with adverse "side effects"; hydrocephalus was induced with high frequency by an undefined mechanism. However, inactivation of Gadd45a did not cause adverse effects and, thus, based on the preclinical studies described here, would constitute a valid target for enhancement of radiation therapy. These observations underscore the need for target validation in specific tumor types using appropriate preclinical models. Finally, in the prostate cancer model, Gadd45a inactivation caused increased apoptosis in the absence of IR (Fig. 3*B*), although the oncogene-induced cell death in this tissue is *p53* independent (18). This unanticipated result suggests that inhibition of Gadd45a alone in some tumor types may have significant antitumor activity. In future experiments, it will be important to test whether *gadd45a* inactivation–mediated sensitization is also effective in other cancer types, especially in those cancers for which surgery or chemotherapy has only modest effects.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Gudkov AV, Komarova EA. The role of p53 in determining sensitivity to radiotherapy. Nat Rev Cancer 2003;3:117–29.
2. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature 2000;408:307–10.
3. Vousden KH, Lu X. Live or let die: the cell's response to p53. Nat Rev Cancer 2002;2:594–604.
4. Smith ML, Ford JM, Hollander MC, et al. p53-mediated DNA repair responses to UV radiation: studies of mouse cells lacking p53, p21, and/or gadd45 genes. Mol Cell Biol 2000;20:3705–14.
5. Wang XW, Zhan Q, Coursen JD, et al. GADD45 induction of a G_2/M cell cycle checkpoint. Proc Natl Acad Sci U S A 1999;96:3706–11.
6. Jin S, Antinore MJ, Lung FD, et al. The GADD45 inhibition of Cdc2 kinase correlates with GADD45-mediated growth suppression. J Biol Chem 2000;275:16602–8.
7. Hollander MC, Philburn RT, Patterson AD, Wyatt MA, Fornace AJ, Jr. Genomic instability in Gadd45a$^{-/-}$ cells is coupled with S-phase checkpoint defects. Cell Cycle 2005;4:704–9.
8. Hollander MC, Sheikh MS, Bulavin DV, et al. Genomic instability in Gadd45a-deficient mice. Nat Genet 1999;23:176–84.
9. Salvador JM, Hollander MC, Nguyen AT, et al. Mice lacking the p53-effector gene Gadd45a develop a lupus-like syndrome. Immunity 2002;16:499–508.
10. Salvador JM, Mittelstadt PR, Belova GI, Fornace AJ, Jr., Ashwell JD. The autoimmune suppressor Gadd45α inhibits the T cell alternative p38 activation pathway. Nat Immunol 2005;6:396–402.
11. Harkin DP, Bean JM, Miklos D, et al. Induction of GADD45 and JNK/SAPK-dependent apoptosis following inducible expression of BRCA1. Cell 1999;97:575–86.
12. Tran H, Brunet A, Grenier JM, et al. DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein. Science 2002;296:530–4.
13. Hildesheim J, Bulavin DV, Anver MR, et al. Gadd45a protects against UV irradiation-induced skin tumors, and promotes apoptosis and stress signaling via MAPK and p53. Cancer Res 2002;62:7305–15.
14. Tront JS, Hoffman B, Liebermann DA. Gadd45a suppresses Ras-driven mammary tumorigenesis by activation of c-Jun NH_2-terminal kinase and p38 stress signaling resulting in apoptosis and senescence. Cancer Res 2006;66:8448–54.
15. Tong T, Ji J, Jin S, et al. Gadd45a expression induces Bim dissociation from the cytoskeleton and translocation to mitochondria. Mol Cell Biol 2005;25:4488–500.
16. Saenz Robles MT, Symonds H, Chen J, Van Dyke T. Induction versus progression of brain tumor development: differential functions for the pRB- and p53-targeting domains of simian virus 40 T antigen. Mol Cell Biol 1994;14:2686–98.
17. Symonds H, Krall L, Remington L, et al. p53-dependent apoptosis suppresses tumor growth and progression *in vivo*. Cell 1994;78:703–11.
18. Hill R, Song Y, Cardiff RD, Van Dyke T. Heterogeneous tumor evolution initiated by loss of pRb function in a preclinical prostate cancer model. Cancer Res 2005;65:10243–54.
19. Brugarolas J, Chandrasekaran C, Gordon JI, Beach D, Jacks T, Hannon GJ. Radiation-induced cell cycle arrest compromised by p21 deficiency. Nature 1995;377:552–7.
20. Pan H, Yin C, Dyson NJ, Harlow E, Yamasaki L, Van Dyke T. Key roles for E2F1 in signaling p53-dependent apoptosis and in cell division within developing tumors. Mol Cell 1998;2:283–92.
21. Chen JD, Van Dyke T. Uniform cell-autonomous tumorigenesis of the choroid plexus by papovavirus large T antigens. Mol Cell Biol 1991;11:5968–76.
22. Wang YA, Elson A, Leder P. Loss of p21 increases sensitivity to ionizing radiation and delays the onset of lymphoma in atm-deficient mice. Proc Natl Acad Sci U S A 1997;94:14590–5.
23. Chan TA, Hwang PM, Hermeking H, Kinzler KW, Vogelstein B. Cooperative effects of genes controlling the G_2/M checkpoint. Genes Dev 2000;14:1584–8.
24. Simin K, Wu H, Lu L, et al. pRb inactivation in mammary cells reveals common mechanisms for tumor initiation and progression in divergent epithelia. PLoS Biol 2004;2:E22.
25. Lu X, Magrane G, Yin C, Louis DN, Gray J, Van Dyke T. Selective inactivation of p53 facilitates mouse epithelial tumor progression without chromosomal instability. Mol Cell Biol 2001;21:6017–30.
26. Santucci MA, Barbieri E, Frezza G, et al. Radiation-induced gadd45 expression correlates with clinical response to radiotherapy of cervical carcinoma. Int J Radiat Oncol Biol Phys 2000;46:411–6.

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