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.

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. 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 14-3-3ε] 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 G2-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 fibroblasts exhibit increased aneuploidy accompanied with abnormal centromere amplification; when exposed to IR, gadd45a knockout mice also show increased lymphohemagogenesis 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 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 TgT121 brain tumor mouse model (16, 17), the TgAPT121 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. TgT121;gadd45a+/− and TgT121;gadd45a−/− were generated by crossing hemizygous TgT121 mice with gadd45a+/− mice, and TgT121;gadd45a−/− were generated by crossing hemizygous TgT121 mice with p21−/− mice. TgAPT121;gadd45a+/− mice were generated by crossing TgAPT121 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 male mice were treated with one 10-Gy dose whole-body radiation and then euthanized 4.5 h after treatment for terminal deoxynucleotidyl 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
10-Gy whole-body dose. For survival analysis, 2-mo-old TgT121;gadd45a/C0 and TgT121;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, TgT121, in which choroid plexus carcinoma (CPC) development is initiated by cell-specific transgenic expression of T121, an NH2-terminal fragment of SV40 T antigen that inactivates pRb and related proteins, p107 and p130 (21). T121 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 TgT121 mice, we generated TgT121;gadd45a/C0 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 gadd45a deficiency, we treated TgT121;gadd45a/C0, TgT121;gadd45a+/+, and TgT121;gadd45a+/+ mice with a single dose of IR to the head (10 Gy) and examined acute...
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Figure 3. Gadd45α 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. Gadd45α deficiency increased the sensitivity of these cells to IR. B, quantitative assessment of data as described in Materials and Methods. Gadd45α 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 TgT121;gadd45α−/− tumors (16.5 ± 3.6%; n = 5) compared with the TgT121;gadd45α+/− controls (8.2 ± 0.8%; n = 5; P < 0.05). TgT121;gadd45α+/− tumors yielded an intermediate apoptosis index (12.4 ± 2.7%; n = 4; Fig. 2). The CPE of nontransgenic mice, both gadd45α+/− and gadd45α−/−, 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 G1 or G2-M cell cycle arrest (19, 22, 23). Thus, we also examined the IR-induced apoptosis in CPE tumors of TgT121;p21+/− mice. Similar to that of TgT121;gadd45α−/− mice, without IR treatment the average apoptosis index of TgT121;p21−/− mice was about the same as that of TgT121;p21+/+ mice. However, with IR treatment the average apoptosis index in tumors of TgT121;p21−/− mice (17.1 ± 2.3%; n = 3) was ~2-fold greater than that of TgT121;p21+/+ mice (8.5 ± 0.8%; n = 5; P < 0.05), with an intermediate level of apoptosis in the tumors of TgT121;p21−/− mice (11.5 ± 1.7%; n = 4; Fig. 2). Inactivating both gadd45α and p21 genes caused an even higher level of IR-induced apoptosis (21.1 ± 1.1%; n = 5) compared with inactivating either gadd45α or p21 alone (Fig. 2). Although the apoptosis level was significantly increased, there was no significant change in the tumor cell proliferation rates in TgT121;gadd45α−/− and TgT121;p21−/− mice compared with TgT121 control mice as determined by BrdUrd incorporation (data not shown).

These data indicate that inactivation of p53 downstream cell cycle arrest genes gadd45α 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 TgT121;p53+/− and TgT121;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 TgT121;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, TgAPT121. In this model, tumors were initiated by prostate epithelial expression of T121 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 T121-induced mammary gland tumor model (17, 24), the apoptosis is not mediated by p53 but rather by phosphatase and tensin homologue (18). TgAPT121 male mice display slow progression to well-differentiated prostate adenocarcinoma (18). We generated TgAPT121;gadd45α−/−, TgAPT121;gadd45α−/−, and TgAPT121;p21−/−;gadd45α−/− 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. 3A). TgAPT121;gadd45α−/− prostate apoptosis increased to 10.0 ± 1.7% (n = 6; P < 0.05; Fig. 3B), whereas TgAPT121;gadd45α−/− prostates showed intermediate levels of apoptosis (14.9 ± 2.6%). Once again, gadd45α 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 gadd45α sensitizes prostate cancer cells to IR in vivo. It is worth to note that in the absence of IR, gadd45α deficiency also caused increased apoptosis level without IR.

Because apoptosis levels are a critical factor in overall tumor growth rates and animal survival, we further examined the effect of
gadd45a inactivation on the survival of IR-treated mice. Brain carcinomas of TgT121 mice do not reproducibly affect survival (18, 25). Therefore, the brain tumor model was used for survival studies. In the absence of IR, TgT121;gadd45a+/– mice had a shorter survival time (t50 = 207 days; n = 45) compared with TgT121;gadd45a–/– mice (t50 = 263 days; n = 60). P < 0.05, log-rank test. B, after sublethal IR treatment to the head (see Materials and Methods), TgT121;gadd45a+/– mice had a better survival (t50 = 285 days; n = 7) than TgT121;gadd45a+/+ mice (t50 = 223 days; n = 18; P < 0.05, log-rank test).

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 TgT121;p21+/– mice compared with TgT121;p21+/+ mice, the effect was negated on further deficiency in p53 (Fig. 2B). 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. 3B), 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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Figure 4. Gadd45a inactivation extended survival from brain tumors in IR-treated TgT121 mice. A, survival of TgT121;gadd45a+/+ mice and TgT121;gadd45a–/– mice without IR treatment. Kaplan-Meier curves showed a shorter survival of TgT121;gadd45a–/– mice (t50 = 207 days; n = 45) compared with TgT121;gadd45a+/+ mice (t50 = 263 days; n = 60; P < 0.05, log-rank test). B, after sublethal IR treatment to the head (see Materials and Methods), TgT121;gadd45a+/– mice had a better survival (t50 = 285 days; n = 7) than TgT121;gadd45a+/+ mice (t50 = 223 days; n = 18; P < 0.05, log-rank test).
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