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Dimethylbenzanthracene Carcinogenesis in *Gadd45a*-null Mice Is Associated with Decreased DNA Repair and Increased Mutation Frequency

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ABSTRACT

Mice lacking the *Gadd45a* gene are susceptible to ionizing radiation-induced tumors. Increased levels of *Gadd45a* transcript and protein are seen after treatment of cells with ionizing radiation as well as many other agents and treatments that damage DNA. Because cells deficient in *Gadd45a* were shown to have a partial defect in the global genomic repair component of the nucleotide excision repair pathway of UV-induced photoproducts, dimethylbenzanthracene (DMBA) carcinogenesis was investigated because this agent produces bulky adducts in DNA that are also repaired by nucleotide excision repair. Wild-type mice and mice deficient for *Gadd45a* were injected with a single i.p. dose of DMBA at 10–14 days of age. The latency for spontaneous deaths was slightly decreased for *Gadd45a*-null mice compared with wild-type mice. At 17 months, all surviving animals were killed, and similar percentages of each genotype were found to have tumors. However, nearly twice as many *Gadd45a*-null than wild-type mice had multiple tumors, and three times as many had multiple malignant tumors. The predominant tumor types in wild-type mice were lymphoma and tumors of the intestines and liver. In *Gadd45a*-null mice, there was a dramatic increase in female ovarian tumors, male hepatocellular tumors, and in vascular tumors in both sexes. In wild-type mice, this dose of DMBA induced a >5-fold increase in *Gadd45a* transcript in the spleen and ovary, whereas the increase in liver was >20-fold. Nucleotide excision repair, which repairs both UV- and DMBA-induced DNA lesions, was substantially reduced in *Gadd45a*-null lymphoblasts. Mutation frequency after DMBA treatment was threefold higher in *Gadd45a*-null liver compared with wild-type liver. Therefore, lack of basal and DMBA-induced *Gadd45a* may result in enhanced tumorigenesis because of decreased DNA repair and increased mutation frequency. Genomic instability, decreased cell cycle checkpoints, and partial loss of normal growth control in cells from *Gadd45a*-null mice may also contribute to this process.

INTRODUCTION

Gadd45a is a M_r 21,000 protein that has been linked to many important cellular processes such as DNA repair, chromatin accessibility, cell cycle checkpoints, and genome stability (1–5). GGR[2], a subtype of NER was decreased in mouse embryo fibroblast cells lacking *Gadd45a*, whereas TCR was unchanged (2). *In vitro* assays have shown that addition of Gadd45a can prevent assembly of histones onto DNA and can specifically bind to UV-induced lesions in nucleosome complexes (3). It was, therefore, proposed that Gadd45a may enhance DNA repair by allowing accessibility of repair complexes to damaged DNA.

In the human genetic diseases XP and CS, one of many genes involved in NER, which confers sensitivity of these individuals to UV radiation-induced carcinogenesis and/or sunburn, is mutated or missing (reviewed in Ref. 6). Mouse models of XP have been generated that show this same predisposition to UV-induced carcinogenesis as well as carcinogenesis by other agents that produce damage repaired by NER (7, 8). The NER defects in XP often involve the GGR subpathway and are associated with increased tumors. In contrast, a defect in TCR, such as in CS, is associated with sun sensitivity, yet no increased carcinogenesis has been observed (9). GGR is defective in *Gadd45a*-null cells, and therefore, increased carcinogenesis by agents producing damage repaired by NER was anticipated in mice lacking this gene.

Gadd45a-null mice were shown to have a decreased latency for IR-induced tumors (5). However, IR produces predominantly DNA strand breaks and base damage that are not repaired by NER. Like XPA and XPC-null mice, most *Gadd45a*-null mice appear normal and do not show a significant increase in spontaneous tumors. However, laboratory mice live in very controlled environments in the absence of UV radiation or carcinogens. It is likely that these genes are not required to prevent spontaneous tumors when there is no exogenous damage to the DNA. In the presence of damage, lack of any one of many DNA repair genes could lead to increased mutagenesis and consequently carcinogenesis. To determine the effect of *Gadd45a* deletion on tumorigenesis by an agent whose damage is repaired by NER, young mice were injected i.p. with DMBA and monitored for tumors. There was a small increase in tumor-induced mortality and prevalence of tumors in *Gadd45a*-null mice compared with wt mice. However, there were far more *Gadd45a*-null mice with multiple tumors and a dramatic increase in vascular, ovarian, and hepatocellular tumors.

MATERIALS AND METHODS

Animals. Mice were housed in Plexiglas cages and given autoclaved NIH 31 diet and water *ad libitum.* NIH is an Association for Assessment and Accreditation of Laboratory Animal Care-accredited animal facility, and all experiments were done under an approved NCI animal study protocol.

Mice were treated at 10–14 days of age with a single i.p. injection of DMBA in corn oil. The study consisted of 11 female and 15 male *Gadd45a*-null mice and 11 female and 13 male wt mice. Animals exhibiting obvious tumors or who were moribund, cachectic, or nonresponsive were killed for necropsy, and at 17 months after DMBA injection, surviving animals were killed for necropsy. Tumors and abnormal tissue were taken for histopathological analysis. Tumor and tissue sections were collected, stained with H&E, and evaluated by a board-certified veterinary pathologist.

DNA Repair and Mutation Assays. Splenic lymphocytes were isolated from spleens of 4- to 6-week-old mice by disruption between two sterile glass slides and grown as previously described (5). The relative percentage of 6-4 photoproducts in total genomic DNA was determined using an ELISA as previously described (10, 11). Briefly, splenic cells in culture were UV (254 nm) irradiated (10 J/m^2), and DNA was prepared 3 to 24 h later. Genomic DNA was extracted using a Blood Kit (Qiagen) according to the manufacturer's recommendations. Polyvinyl chloride flat-bottomed microtiter plates precoated with 1% protamine sulfate (Sigma) were incubated with 300 ng of DNA in PBS at 37°C for 20 h. After drying, the plates were washed five times with PBS containing 0.05% Tween (PBST). The plates were blocked with 2% fetal bovine serum in PBS for 30 min at 37°C. After five washings with PBST, the plates were incubated with 64M-2 (11) anti-(6-4) photoproduct antibodies (in

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[2] The abbreviations used are: GGR, global genomic repair; NER, nucleotide excision repair; TCR, transcription-coupled repair; CS, Cockayne's syndrome; XP, xeroderma pigmentosum; IR, ionizing radiation; DMBA, dimethylbenzanthracene; wt, wild type.

quadruplicate) diluted 1:1000 in PBS. Another five PBST washings were followed by two consecutive incubations (30 min, 37°C) with goat antimouse IgG $F(ab')_2$ fragment conjugated with biotin (1:2000 dilution in PBS; Zymed) and then with streptavidin-peroxidase conjugate (1:10000 dilution in PBS; Zymed). Finally, after five PBST washings and one citrate-phosphate buffer (pH 5.0) washing, 100 μl of substrate solution (0.04% *o*-phenylene diamine and 0.0075% H_2O_2 in citrate-phosphate buffer) were added to each well and incubated for 30 min at 37°C. Reactions were stopped with 50 μl 2 M H_2SO_4. The absorbance at 490 nm was measured using an E-max microplate reader (Molecular Devices).

Gadd45a-null mice were crossed into the BigBlue strain of mice, which harbor a λ shuttle vector that can be used for mutation detection. Six-week-old mice that carried the λ integration and were either wt or null for *Gadd45a* were injected i.p. with 20 nmol/g body weight DMBA, and tissues were harvested for DNA isolation 7 days later. Genomic DNA was prepared using the RecoverEase DNA isolation kit, and bacteriophage were packaged using Transpack packaging extract (Stratagene). At least 2×10^4 plaques were screened for λcII mutations, according to the manufacturer's instructions in the λcII Mutation Assay Detection Kit (Stratagene).

RNA Analysis. For RNA isolation, 6-week-old mice were injected i.p. with 20 nmol of DMBA/g body weight, and tissues were harvested 4 h later. Tissues were immediately homogenized in RNAzol (Life Technologies), and RNA was prepared as directed by the manufacturer. RNA was dot-blotted onto nylon membranes (Nytran; Schleicher & Schull). cDNA probes were labeled with [^{32}P]dCTP using Prime-It random primer labeling (Stratagene). *Gadd45a* RNA was normalized to poly(A) content, which was estimated by hybridization to a labeled polyuridylic acid probe (12, 13). Radioactivity for RNA dot blots was counted on a PhosphorImager (Molecular Dynamics).

RESULTS

Because *Gadd45a* is involved in NER, it was expected that there would be decreased DNA repair leading to increased DMBA-induced mutations in *Gadd45a*-null animals, resulting in more tumors. Because of the involvement of Gadd45a in cell cycle checkpoints, growth control, and genome stability, there was also the possibility that these features of the *Gadd45a*-null phenotype could contribute to decreased latency and increased incidence. The dose of DMBA used was expected to produce tumors in wt animals so that tumor types, incidence rates, and latency could be compared between *Gadd45a*-null and wt mice. At 16 months posttreatment, there was a slight increase in the incidence of deaths in the *Gadd45a*-null mice (Fig. 1), primarily attributable to killing of moribund *Gadd45a*-null males. For

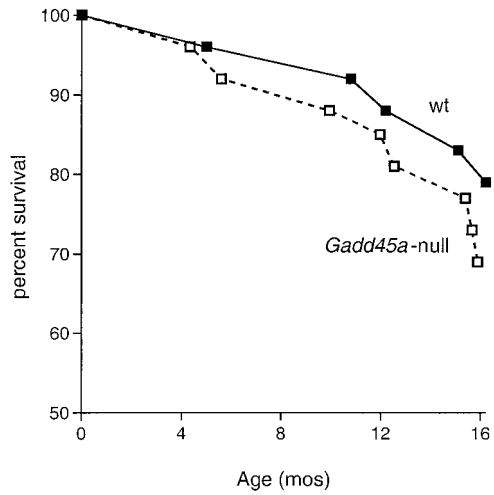

Fig. 1. Survival of *Gadd45a*-null and wt mice after DMBA treatment. Mice were injected at 10–14 days old with 20 nmol/g body weight i.p. DMBA. Animals were followed for up to 17 months at which time all surviving animals were necropsied. Mice with obvious tumors or ill health were killed for necropsy, and most mice that died before 17 months were found to have tumors.

males, 6 (40%) of 15 *Gadd45a*-null died early compared with 2 (15%) of 13 wt, whereas in the females, 3 (27%) of 11 *Gadd45a*-null died early compared with 4 (36%) of 11 wt. Only one early killing each in the wt males and *Gadd45a*-null males and two early sacrifices in the *Gadd45a*-null females were not attributable to neoplasia. All surviving mice were necropsied at 17 months.

Total tumor burden was greater in the *Gadd45a*-null mice of both sexes than in wt mice (Table 1). This increase was attributable to a greater number of male *Gadd45a*-null mice with neoplasm as well as to increased multiplicity of tumors per animal, both benign and malignant, in both the male and female *Gadd45a*-null when compared with the wt animals. Almost twice as many *Gadd45a*-null had more than one tumor, and more than three times as many *Gadd45a*-null mice had two or more malignant tumors (malignancy being based on presence of metastasis, invasion, or atypia), when compared with the wt mice (Fig. 2). The average number of all tumors per affected mouse was 1.53 and 2.37 for wt and *Gadd45a*-null mice, respectively [significantly different ($P < 0.03$) by *t* test and one-way ANOVA].

There was a notable increase in incidence of hepatocellular tumors in the male *Gadd45a*-null, ovarian tumors in the female *Gadd45a*-null, and hemangiomas/hemangiosarcomas in both male and female *Gadd45a*-null mice when compared with the wt (Table 1). Hemangiomas/hemangiosarcomas were most prevalent in the intestinal tract of both *Gadd45a*-null and wt mice; however, in the *Gadd45a*-null mice these vascular tumors tended to occur in multiple sites and in additional tissues including the spleen, pancreas, adipose tissue, and skeletal muscle.

Gadd45a RNA is increased in cell cultures and *in vivo* after treatment with a variety of agents that damage DNA. This increase might provide a protective function because Gadd45a is involved, either directly or indirectly, in NER, the DNA repair pathway that repairs bulky lesions such as those produced by DMBA. Wt mice were injected with the same dose of DMBA that was used for carcinogenesis, and *Gadd45a* RNA levels were measured after 4 h, the time when *Gadd45a* RNA levels are often maximal after treatment with DNA-damaging agents. From 5- to >20-fold induction was seen in various tissues (Fig. 3). The highest induction was seen in the liver, which might be expected because this is probably the major organ that metabolizes DMBA. Induction was seen both in organs in which tumors were more prevalent in *Gadd45a*-null mice as well as those in which there was no increase in tumors in *Gadd45a*-null mice.

Decreased repair has been shown in *Gadd45a*-null mouse embryo fibroblasts (2). To determine whether repair is attenuated in tissues from *Gadd45a*-null mice, splenic lymphocytes were treated with UV radiation and allowed to repair the DNA damage for up to 24 h. Although UV and DMBA produce different types of DNA lesions, they are repaired by the same mechanism, NER. UV radiation was used here instead of DMBA because antibodies to 6-4 UV photoproducts are available to measure DNA lesions. As predicted, repair was diminished in *Gadd45a*-null lymphocytes compared with wt lymphocytes (Fig. 4).

Decreased repair of DMBA lesions would be expected to result in increased mutations, which might lead to tumor formation. *Gadd45a*-null mice were bred into the BigBlue mouse strain, which uses a bacteriophage λ shuttle vector for mutation detection. Livers were taken from DMBA-treated mice 7 days after treatment, and mutation frequencies of the λ cII gene were determined (Fig. 5). *Gadd45a*-null livers exhibited three times more DMBA-induced mutations than wt livers. *Gadd45a*-null and wt livers showed a 3.1- and 2.3-fold increase in mutations after DMBA treatment, respectively. Basal mutation frequency in wt livers was similar to published data but was increased 1.7-fold in *Gadd45a*-null livers.

Table 1 *Tumor types and location in DMBA-treated mice*

	Wt male	Null male[a]	Wt female	Null female
No. of mice	13	15	11	11
Total number of tumors	11	26	14	22
No. of mice with any tumor (%)	7 (54)	12 (80)	8 (73)	7 (64)
No. of mice with >1 tumor (%)	3 (23)	9 (60)	4 (36)	6 (55)
No. of mice with >1 malignancy (%)	2 (15)	6 (40)	1 (9)	4 (36)
Hemangioma (B)				
Liver	0	3	1	1
Uterus	—	—	3	2
Pancreas	0	0	0	1
Intestine	0	0	0	1
Hemangiosarcoma (M)				
Intestine	5	5	3	5
Spleen	0	3	0	1
Liver	0	1	0	0
Uterus	—	—	0	1
Adipose tissue	0	0	0	1
Skeletal muscle	0	0	0	1
Total vascular tumors (%)[b]	5 (38)	12 (80)	7 (64)	14 (127)
Ovary				
Granulosa cell (B)	—	—	0	4
Granulosa cell (M)	—	—	1	0
Cystadenoma (B)	—	—	0	1
Total ovarian tumors (%)[b]			1 (9)	5 (45)
Liver				
Hepatocellular adenoma (B)	1	2	1	1
Hepatocellular sarcoma (M)	1	3	0	0
Total hepatocellular tumors (%)[b]	2 (15)	5 (33)		
Hematopoietic neoplasm (M)				
FCC	1	1	3	1
Histiocytic sarcoma	0	2	0	0
Lung				
Alveolar adenoma (B)	1	2	0	0
Alveolar carcinoma (M)	2	2	0	0
Skin				
Keratoacanthoma (B)	0	1	0	0
Sarcoma (M)	0	0	2	1
Epididymis				
Mesothelium (M)	0	1	0	0

[a] Null, *Gadd45a*-null; B, benign; M, malignant.
[b] Values are the total number of tumors divided by number of mice for that group. Numbers above 100% indicate more than one of that tumor type per mouse.

DISCUSSION

Many human tumor-prone disorders result from mutation of known DNA repair genes. In many cases, loss of DNA repair capacity is associated with family history of specific tumor types, such as mismatch repair defects in hereditary nonpolyposis colon cancer (14). In some cases, tumors are known to result from environmental exposure, such as UV radiation-induced tumors in XP patients (9). In addition, mouse models of XP show increased tumorigenesis by other agents that produce damage repaired by NER (7, 8). However, not all DNA repair defects lead to increased tumorigenesis. For example, the CS group B gene (*CSB*) is involved in transcription-coupled repair, but patients with this disorder do not develop UV radiation-induced tumors, possibly because of increased apoptosis of affected cells (15). *Gadd45a*-null cells have a partial defect in the GGR subpathway of NER (2), similar to that observed in XP group C and group E mutants (9). Mice lacking *Gadd45a* develop ionizing radiation-induced tumors, although the DNA lesions produced are not repaired by GGR. The mechanism for this tumorigenesis is unknown but may be related to the induction of *Gadd45a* by IR in a p53-dependent fashion. In addition, Gadd45a may be involved in other pathways of DNA repair

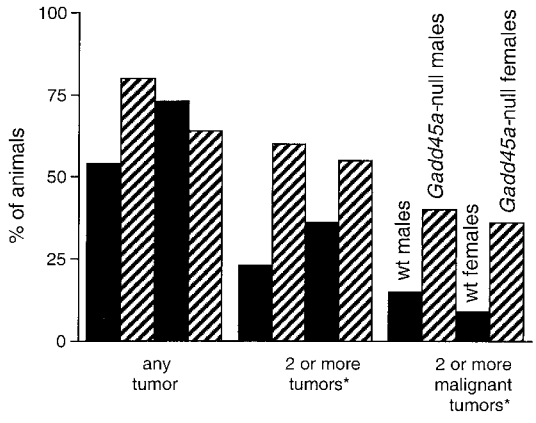

Fig. 2. DMBA-induced tumor incidence in *Gadd45a*-null and wt mice. Animals are the same as those in Fig. 1. Data include tumors from animals that died during the study as well as animals killed at 17 months at the end of the study. Tumors were examined and typed by a board-certified veterinary pathologist. *, significantly different ($P < 0.03$) by *t* test and one-way ANOVA.

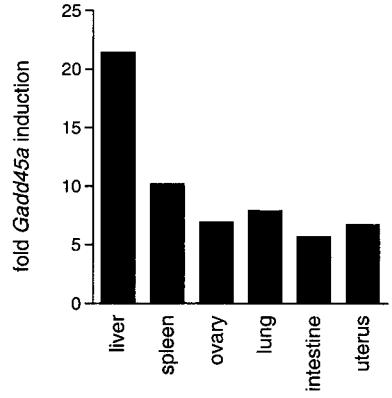

Fig. 3. Gadd45a RNA levels after injection with 20 nmol/g body weight i.p. DMBA. Female mice were injected at 6 weeks of age, and tissues were harvested for RNA 4 h after treatment, as described in "Materials and Methods." Results are average from three untreated and three treated animals.

because a role for the Gadd45a protein was proposed in chromatin accessibility to repair complexes (3).

DMBA produces bulky DNA adducts that are repaired via NER. Because NER is defective in cells derived from *Gadd45a*-null mice, it was expected that unrepaired DMBA mutations would lead to more tumors in these mice. *Gadd45a*-null mice did indeed develop more DMBA-induced tumors than wt mice (Fig. 2). This increase in tumors was associated with decreased NER in splenic lymphocytes (Fig. 4), and an increase in DMBA-induced mutations was found in *Gadd45a*-null liver, consistent with decreased repair (Fig. 5). Therefore, a mechanism for increased tumorigenesis in these mice can be ascertained by which critical mutations lead to tumor formation.

XP and CS are two rare human photosensitive disorders (9). Nine different genes were found to be responsible for various forms of these diseases, all of which are involved in NER. *XPA*, *XPB*, *XPD*, *XPF*, and *XPG* are all defective in both subpathways of NER, GGR, and TCR. Only *XPC* and *XPE* are involved solely in GGR. In contrast, *CSA* and *CSB* are involved solely in TCR. In animal models, GGR is the major determinant of UV-induced skin cancer, whereas TCR is the major determinant of sunburn (16). This is consistent with the relative lack of tumor formation in CS patients, whose cells are defective only in TCR. Like *XPC*, Gadd45a is not required for efficient TCR but is essential for maximal GGR.

Other similarities exist between *XPC*, *XPE*, and Gadd45a. All three have affinity for UV-induced DNA lesions in the context of chromatin and have postulated roles in lesion accessibility. *XPC* is the earliest factor involved in the initial recognition of damage in reconstituted *in vitro* assays. *XPC* changes the DNA conformation around lesions, and this has been suggested to facilitate binding of other NER proteins to the lesion (17). Likewise, Gadd45a can bind to damaged DNA in chromatin, the natural state of DNA in the cell. *In vitro* experiments suggest that Gadd45a may alter chromatin structure, perhaps allowing access to *XPC* or other repair proteins *in vivo*. *XPE* forms a complex *in vivo* that tightly associates with chromatin after DNA damage, suggesting that, like Gadd45a, it is also involved in recognition of chromatinized DNA damage (18). In addition to established or potential roles in damage recognition, *XPC*, *XPE*, and Gadd45a are all activated by the tumor suppressor p53 (19, 20).[3] p53 regulation of these three genes, which may have similar functions in damage recognition, supports the involvement of p53 in the NER pathway.

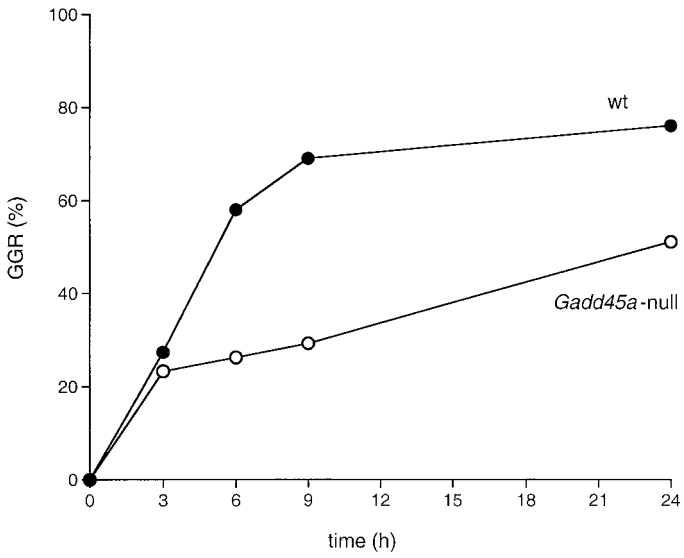

Fig. 4. GGR of 6-4 photoproducts in *Gadd45a*-null and wt primary mouse splenic lymphocytes. Results are representative of two experiments (two wt and two *Gadd45a*-null mice), which showed decreased repair in *Gadd45a*-null compared with wt lymphocytes.

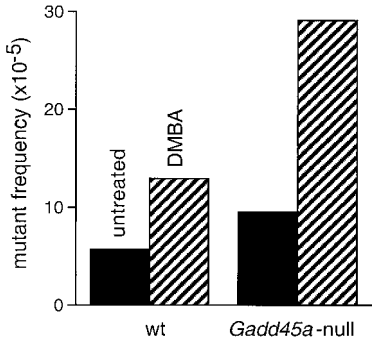

Fig. 5. Increased persistent DMBA-induced mutations in liver of *Gadd45a*-null mice. Male and female BigBlue transgenic mice of the indicated genotypes were treated as in Fig. 3, and liver DNA was isolated 7 days after treatment. Mutations in the λ cII gene were detected, as described in "Materials and Methods."

XPA-deficient mice are sensitive to UV-induced skin tumors as well as to benzo(*a*)pyrene-induced internal tumors (7, 21). Like *Gadd45a*-null mice, none of these mouse models for human NER deficiency syndromes show high levels of spontaneous tumors. *Gadd45a* deletion in mice, therefore, resembles mouse models of XP. Mouse models of XP, however, have generally less severe phenotypes than their human counterparts, which also show neurological symptoms (9). This is not surprising because GGR in mice is much less robust than in humans. Human mutations in *Gadd45a* have not been found in human tumor cell lines (Ref. 22 and data not shown), although few tumor types have been examined. A human inactivation of *Gadd45a* would be expected to confer increased carcinogen-induced tumorigenesis, perhaps similar to XP.

DMBA-treated *Gadd45a*-null mice had a dramatically higher multiplicity of tumors than did wt mice, with many developing multiple different malignant tumors. The reason for this increase in tumorigenicity and malignancy may result from the growth and transformation properties observed for *Gadd45*-null cells in culture. *Gadd45a*-null MEF grow more rapidly and are immortal. These cells are transformed by a single oncogene (activated ras) and exhibit genomic instability (5). Therefore, in a multistage model of carcinogenesis, *Gadd45a*-null cells may already have compromised growth control mechanisms and hence may be even more susceptible to malignant transformation by additional cellular events.

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REFERENCES

1. Smith, M. L., Chen, I. T., Zhan, Q., Bae, I., Chen, C. Y., Gilmer, T. M., Kastan, M. B., O'Connor, P. M., and Fornace, A. J., Jr. Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen [see comments]. Science (Washington DC), *266:* 1376–1380, 1994.
2. Smith, M. L., Ford, J. M., Hollander, M. C., Bortnick, R. A., Amundson, S. A., Seo, Y. R., Deng, C. X., Hanawalt, P. C., and Fornace, A. J., Jr. p53-mediated DNA repair responses to UV radiation: studies of mouse cells lacking *p53, p21,* and/or *gadd45* genes. Mol. Cell. Biol., *20:* 3705–3714, 2000.
3. Carrier, F., Georgel, P. T., Pourquier, P., Blake, M., Kontny, H. U., Antinore, M. J., Gariboldi, M., Myers, T. G., Weinstein, J. N., Pommier, Y., and Fornace, A. J., Jr. Gadd45, a p53-responsive stress protein, modifies DNA accessibility on damaged chromatin. Mol. Cell. Biol., *19:* 1673–1685, 1999.

[3] Sally Amundson, personal communication.

4. Zhan, Q., Antinore, M. J., Wang, X. W., Carrier, F., Smith, M. L., Harris, C. C., and Fornace, A. J., Jr. Association with Cdc2 and inhibition of Cdc2/Cyclin B1 kinase activity by the p53-regulated protein Gadd45. Oncogene, *18:* 2892–2900, 1999.
5. Hollander, M. C., Sheikh, M. S., Bulavin, D. V., Lundgren, K., Augeri-Henmueller, L., Shehee, R., Molinaro, T. A., Kim, K. E., Tolosa, E., Ashwell, J. D., Rosenberg, M. P., Zhan, Q., Fernandez-Salguero, P. M., Morgan, W. F., Deng, C. X., and Fornace, A. J., Jr. Genomic instability in Gadd45a-deficient mice. Nat. Genet., *23:* 176–184, 1999.
6. de Boer, J., and Hoeijmakers, J. H. Nucleotide excision repair and human syndromes. Carcinogenesis (Lond.), *21:* 453–460, 2000.
7. de Vries, A., van Oostrom, C. T., Hofhuis, F. M., Dortant, P. M., Berg, R. J., de Gruijl, F. R., Wester, P. W., van Kreijl, C. F., Capel, P. J., van Steeg, H., *et al.* Increased susceptibility to ultraviolet-B and carcinogens of mice lacking the DNA excision repair gene *XPA*. Nature (Lond.), *377:* 169–173, 1995.
8. Friedberg, E. C., Bond, J. P., Burns, D. K., Cheo, D. L., Greenblatt, M. S., Meira, L. B., Nahari, D., and Reis, A. M. Defective nucleotide excision repair in xpc mutant mice and its association with cancer predisposition. Mutat. Res., *459:* 99–108, 2000.
9. van Steeg, H., and Kraemer, K. H. Xeroderma pigmentosum and the role of UV-induced DNA damage in skin cancer. Mol. Med. Today, *5:* 86–94, 1999.
10. Matsunaga, T., Mori, T., and Nikaido, O. Base sequence specificity of a monoclonal antibody binding to (6-4) photoproducts. Mutat. Res., *235:* 187–194, 1990.
11. Mori, T., Nakane, M., Hattori, T., Matsunaga, T., Ihara, M., and Nikaido, O. Simultaneous establishment of monoclonal antibodies specific for either cyclobutane pyrimidine dimer or (6-4) photoproduct from the same mouse immunized with ultraviolet-irradiated DNA. Photochem. Photobiol., *54:* 225–232, 1991.
12. Hollander, M. C., and Fornace, A. J., Jr. Estimation of relative mRNA content by filter hybridization to a polythymidylate probe. Biotechniques, *9:* 174–179, 1990.
13. Koch-Paiz, C. A., Momenan, R., Amundson, S. A., Lamoreaux, R., and Fornace, A. J., Jr. Estimation of relative mRNA content by filter hybridization to a polyuridylic probe. Biotechniques, *29:* 706–714, 2000.
14. Buermeyer, A. B., Deschenes, S. M., Baker, S. M., and Liskay, R. M. Mammalian DNA mismatch repair. Annu. Rev. Genet., *33:* 533–564, 1999.
15. Ljungman, M., and Zhang, F. Blockage of RNA polymerase as a possible trigger for u.v. light-induced apoptosis. Oncogene, *13:* 823–831, 1996.
16. Berg, R. J., Rebel, H., van der Horst, G. T., van Kranen, H. J., Mullenders, L. H., van Vloten, W. A., and de Gruijl, F. R. Impact of global genome repair versus transcription-coupled repair on ultraviolet carcinogenesis in hairless mice. Cancer Res., *60:* 2858–2863, 2000.
17. van der Spek, P. J., Eker, A., Rademakers, S., Visser, C., Sugasawa, K., Masutani, C., Hanaoka, F., Bootsma, D., and Hoeijmakers, J. H. XPC and human homologs of RAD23: intracellular localization and relationship to other nucleotide excision repair complexes. Nucleic Acids Res., *24:* 2551–2559, 1996.
18. Otrin, V. R., McLenigan, M., Takao, M., Levine, A. S., and Protic, M. Translocation of a UV-damaged DNA binding protein into a tight association with chromatin after treatment of mammalian cells with UV light. J. Cell Sci., *110* (Pt 10):1159–1168, 1997.
19. Kastan, M. B., Zhan, Q., el-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B., and Fornace, A. J., Jr. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell, *71:* 587–597, 1992.
20. Hwang, B. J., Ford, J. M., Hanawalt, P. C., and Chu, G. Expression of the p48 xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair. Proc. Natl. Acad. Sci. USA, *96:* 424–428, 1999.
21. Ide, F., Iida, N., Nakatsuru, Y., Oda, H., Tanaka, K., and Ishikawa, T. Mice deficient in the nucleotide excision repair gene XPA have elevated sensitivity to benzo[*a*]pyrene induction of lung tumors. Carcinogenesis (Lond.), *21:* 1263–1265, 2000.
22. Blaszyk, H., Hartmann, A., Sommer, S. S., and Kovach, J. S. A polymorphism but no mutations in the *GADD45* gene in breast cancers. Hum. Genet., *97:* 543–547, 1996.