Mammalian GADD34, an Apoptosis- and DNA Damage-inducible Gene*

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The mammalian cellular response to genotoxic stress is a complex process involving many known and probably many as yet unknown genes. Induction of the human DNA damage- and growth arrest-inducible gene, GADD34, by ionizing radiation was only seen in certain cell lines and correlated with apoptosis following ionizing radiation. In addition, the kinetics and dose response of GADD34 to ionizing radiation closely paralleled that of the apoptosis inhibitor, BAX. However, unlike BAX, the GADD34 response was independent of cellular p53 status. The carboxyl terminus of GADD34 has homology with the carboxyl termini of two viral proteins, one of which is known to prevent apoptosis of virus infected cells. The association of GADD34 expression with certain types of apoptosis and its homology with a known apoptosis regulator suggests that GADD34 may play a role in apoptosis as well.

The response to genotoxic stress in mammalian cells is complex and may include several processes, including enhanced DNA repair, transient growth arrest, and/or apoptosis (1–4). The p53 tumor suppressor gene has been shown to be required for a G2 phase arrest following treatment with agents producing DNA strand breaks and other lesions in DNA (1, 5). It may play a role also in cell cycle arrest in the G2 and M phases but is not strictly required (6–8). p53 is required for apoptosis in certain cell types following certain genotoxic signals (9–11). However, other pathways for apoptosis are evident, since cells lacking normal p53 may also undergo apoptosis following various cellular stresses (9, 10). Models have been proposed whereby specific gene products are involved in various aspects of the DNA damage response. However, there are many missing pieces in all of these pathways that may include novel proteins.

The five gadd genes were originally isolated as UV-inducible transcripts in Chinese hamster ovary (CHO)1 cells. All five gadd genes were also found to be inducible by stressful growth arrest and by various types of DNA damage, hence the designation gadd. GADD33, GADD34, GADD45, and GADD153 are expressed in human cells and are inducible by a wide variety of genotoxic agents. The mouse homolog of GADD153, CHOP, has been shown to be a member of the CCAAT enhancer-binding protein family of transcription factors. The protein binds other

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1 The abbreviations used are: CHO, Chinese hamster ovary cells; HSV-1, herpes simplex virus-1; IR, ionizing radiation; MMS, methyl methanesulfonate; GAPDH, glyceraldehyde phosphate dehydrogenase; poly(A), polyadenylated RNA; kb, kilobase pair(s); bp, base pair(s); Gy, gray(s).

CCAAT enhancer-binding protein family members and changes their DNA sequence binding specificity (12). A translocation that leads to a fusion between GADD153 and an RNA-binding protein is associated with myxoid liposarcomas, and this fusion protein shows altered biochemical properties relative to the normal GADD153 protein (13). The GADD45 protein is also a predominantly nuclear protein that binds proliferating cell nuclear protein and whose expression is regulated by the tumor suppressor p53 as mentioned above (14, 15). GADD153, GADD45, and GADD34 all lead to growth inhibition as measured by colony formation when overexpressed in several human cells lines (16). Interestingly, combinations of these three genes, when overexpressed, lead to synergistic or cooperative effects, suggesting that they inhibit growth through different and perhaps complementary pathways. Apoptosis occurs in certain cell types after treatment with genotoxic agents. Since these treatments also induce the gadd genes, it has been suggested that the gadd genes may be involved in apoptosis as well as in growth arrest after DNA damage. The decrease in plating efficiency in response to overexpression of the gadd genes could be due to the stimulation of apoptosis, a permanent inhibition of cell growth, necrosis, or any combination of these.

GADD34 is the human/hamster homolog of the mouse MyD116 cDNA that was subsequently isolated as a primary response transcript expressed during myeloid differentiation of M1 cells (17). Treatment of M1 cells with MMS leads to apoptosis without differentiation, while treatment with interleukin-6 or lung conditioned medium leads to a myeloid terminal differentiation program, which culminates in apoptosis. In mouse M1 cells, both the gadd and the MyD genes respond to MMS with increased mRNA within several hours. Differentiation of M1 cells with interleukin-6 or lung-conditioned medium leads to a rapid increase in MyD gene mRNA, while the gadd response occurs much later at a time that corresponds to the onset of apoptosis. However, gadd34/MyD116 was unique in that it showed a biphasic response, which was suggestive of both the gadd and MyD kinetics (16). Therefore, in this system, increased expression of gadd34/MyD116 coincides with apoptosis and not with differentiation or DNA damage alone.

To further elucidate the regulation of GADD34 expression by DNA damage and possible functions of the GADD34 protein, a human GADD34 cDNA clone and genomic DNA clones for both the human and hamster genes were isolated and characterized. The human gene is inducible by various types of DNA damage and interestingly is induced by ionizing radiation (IR) in cells which undergo apoptosis following this treatment. The predicted human protein is 73.5 kDa and, like GADD45, GADD153, MDM2, and other damage-inducible proteins, is among the most highly negative charged of mammalian proteins (16).

The predicted protein product of the GADD34 gene has a
region of homology with two viral proteins. One of these, the herpes simplex virus-1 (HSV-1) protein g134.5, is required for inhibition of apoptosis of HSV-1-infected neuroblastoma cells (18). GADD34 has appreciable but less homology with the NL protein of the African swine fever virus, whose function is as yet unknown (19). This area of homology is limited to an 84-amino acid stretch at the carboxyl terminus of the two viral proteins and near the carboxyl terminus of the 674-amino acid GADD34. Substitution of the carboxyl terminus of g134.5 in the HSV-1 genome with the corresponding domain from MyD116, the mouse homolog of gadd34, prevented shutoff of protein synthesis and inhibited apoptosis similar to the wild type HSV-1 virus (20). Based on this similarity, and the increased expression of GADD34 in cells treated with agents that elicit apoptosis, it is conceivable that GADD34 may be involved in apoptosis as well.

**FIG. 1.** cDNA and putative protein sequence of human GADD34. The cDNA sequence is indicated on top and corresponds to the insert sequence from pHu34B. The putative protein sequence is indicated below the coding sequences. The protein contains six imperfect repeats of a 40-amino acid sequence, which are underlined with alternating single or double lines.
**EXPERIMENTAL PROCEDURES**

**cDNA and Genomic Clones—** A full-length hamster gadd34 probe, pXR34m, was used to screen a human ZAP XR cDNA library (Stratagene) to isolate a cDNA corresponding to the full-length human GADD34 transcript. This library was made from mRNA from ML-1 cells treated with 20 Gy of ionizing radiation harvested 3.5 h after treatment. A λ CHO FIXII library (Stratagene) was screened for hamster genomic clones. A human genomic clone, p1-1812, was obtained by polymerase chain reaction screening of a P1 library (Genome Systems) with GADD34-specific primers. The 5′′ primer was CH040 (AAGAGG-GAGTGTCTGAAAGAGGGAGGAG), and the 3′′ primer was CH041 (TCCTCCGTGGCTCTATGCTTCC). Several p1-1812 fragments were subcloned into pGEMZf (+).

Sequencing of the inserts of pHu34B and partially of pH34B1 were done using the deoxy chain termination method and by Lark Sequencing Technologies (Houston, TX). Sequence comparisons were done with the Genetics Computer Group Sequence Analysis Software Package (21) and the National Center for Biotechnology Information Blast program (22).

**Plasmid Constructs—** pCMV-hu34S was made by inserting the 2.2-kb Not I/Hind III fragment from pHu34B into pCMV.3. pZipBcl2-neo contains the 2-kb SpI fragment of pRB143 into pCMV.3. pZipBcl2-neo contains the human BCL-2 cDNA ligated into the BamHI site of pZipNeoSVX and was obtained from J. Marie Hardwick (25).

**Cells and Drug Treatments—** ML-1 and WMN cells were grown in RPMI 1640 with 10% fetal bovine serum. A549, Ovcar-4, and RKO cells and was obtained from J. Marie Hardwick (25).

**BCL-2** with the 4-kb pREP4 (Invitrogen) by polymerase chain reaction and blunt end-ligated Not I/Hin cDNA polymerase chain reaction screening of a P1 library (Genome Systems) was made using a 5′ oligonucleotide probe (30).

**RNA Analysis—** For RNA isolation, cells were rinsed briefly with 5m lysis buffer (150-cm tissue culture plate. Total phosphate-buffered saline and were then immediately lysed in RPMI 1640 with 10% fetal bovine serum. A549, Ovcar-4, and RKO cells and was obtained from J. Marie Hardwick (25).

**RESULTS**

**GADD34 cDNA and Predicted Protein—** Based on the number of clones obtained from the human cDNA library screen, the abundance of GADD34 mRNA was estimated to be less than 0.01% of polyadenylated messages in untreated ML-1 cells. Since the GADD34 transcript is 2.4 kb, several cDNA clones of over 2.2 kb were considered to be nearly full length when the poly(A) tail is taken into consideration. The 2300-bp insert of pHu34B was predicted to comprise a nearly full-length GADD34 cDNA. This clone contains the entire open reading frame and 221 bp of 5′ nontranslated sequence (Fig. 1). The 3′-untranslated sequence is 87 bp. The human GADD34 cDNA has 67–68% identity with the mouse (MyD116) and hamster homologs, while the hamster and mouse share 77% identity between them. At the amino acid level, the human protein has 52–55% identity and 67–70% similarity with the rodent homologs, using the GAP program of the Genetics Computer Group Sequence Analysis Software Package (21). The hamster and mouse proteins are 69% identical with 79% similarity. The predicted human GADD34 protein is 674 amino acids with a predicted size of 73.5 kDa and a pI of 4.4. The protein would be expected to have a net charge of −64 and a net charge per amino acid percent of −9.5. This is similar to both hamster and mouse GADD34 proteins and also to protein products from other gadd and related genes (16). The hamster and mouse proteins are expected to be 64.5 and 71.8 kDa, respectively. The human GADD34 protein, like the hamster and mouse homologs, contains a series of repeated sequences in the center portion of the predicted protein. These repeats are 34 amino acids long for the human protein and 40 and 39 for the hamster.

**FIG. 2. Similarity of repeats within the human GADD34 protein.** The four copies of the human repeated amino acid sequence are indicated with their locations. Highlighted amino acids are shared by two or more of the repeats. The lower two lines represent the consensus from these repeats for the hamster and mouse GADD43 proteins.

**FIG. 3. Homology of human GADD34 with viral proteins HSV-1 γ34.5 and ASV NL.** Only the regions of significant homology are shown. The sequence of the human GADD34 protein in on top, with the hamster and murine GADD34 proteins below. The fourth line is the HSV-1 γ34.5 protein and below that the ASV NL protein. The sequences extend to the carboxyl termini for all proteins. Highlighted amino acids are shared by two or more of the proteins shown.

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373-370
364-417
427-460
477-510
533
583
563
526
576
525
149
164
633
563
624

| 533 | HLRKRRPEFDRHDPDSTLDRKARYPVKERSKVRVTHLAVWAGPAQACOCN583human | 583human |
| 483 | HLRRLLTRFQDDPDSTLVRKARYPVKERSKVRVTHLAVWAGPAQACOCN583human |
| 526 | LRLRFKAPFQDDPDSTLVRKARYPVKERSKVRVTHLAVWAGPAQACOCN583human |
| 563 | RALDRDSSFRIRYQDACELSLICTPAARARAXLRNP583human |
| 576 | RALDRDSSFRIRYQDACELSLICTPAARARAXLRNP583human |
| 525 | ARPRRFQDDPDSTLVRKARYPVKERSKVRVTHLAVWAGPAQACOCN583human |
| 149 | QAQPRRFQDDPDSTLVRKARYPVKERSKVRVTHLAVWAGPAQACOCN583human |
| 164 | HLRKRRPEFDRHDPDSTLDRKARYPVKERSKVRVTHLAVWAGPAQACOCN583human |
| 633 | HLRKRRPEFDRHDPDSTLDRKARYPVKERSKVRVTHLAVWAGPAQACOCN583human |
| 563human |
| 590hamster |
| 674human |
| 590hamster |
| 674human |
| 590hamster |
| 674human |
and mouse proteins, respectively. The hamster protein has 3.5 copies, and the mouse protein 4.5 copies of this sequence, while the human protein has 4 copies. The repeats of the mouse and hamster promoters are arranged in tandem, while the human repeats are separated by varying numbers of amino acids (data not shown). The repeats of the hamster and mouse proteins show nearly 100% identity to each other. However, the human repeats show less similarity to each other and to the rodent repeats (Fig. 2). There were two additional sequences in the human cDNA that showed limited similarity with the other human repeats (data not shown).

A search of the data bases for sequences homologous to the human GADD34 yielded two viral proteins, one of which has nearly 50% identity to human GADD34 in a 90-amino acid region (Fig. 3). This protein, herpes simplex virus type-1-infected cell protein 34.5 (34.5 or ICP34.5) was previously known to have homology to the rodent GADD34 sequences (16, 31). The other viral protein, the African swine fever virus NL protein, shows less homology to GADD34, but this is in the same region as the homology to human repeats. In GADD34, this region is the most highly conserved between species with greater than 90% amino acid homology. Several nucleolin proteins were somewhat homologous to GADD34, but this similarity was limited mostly to aspartic acid and glutamic acid residues present at high numbers in both GADD34 and nucleolin (data not shown).

**Gene Organization—GADD34** was found to be a single copy gene as only one band is visualized on genomic Southern blots when human DNA was cut with 6-bp recognition restriction enzymes, and the full-length cDNA was used as probe (data not shown). Genomic clone p1-1812 hybridized to highly stringency with a probe made from the insert in phu34B. Equivalent sized hybridizing bands were observed with p1-1812 and genomic DNA after DNA was cut with the 6-bp recognizing restriction enzymes HindIII, EcoRI, BamHI, XbaI, and KpnI (data not shown). Comparison of genomic and cDNA sequences revealed that the human GADD34 gene has two introns and that translation begins in the second exon (data not shown). To facilitate analysis of proximal genomic sequences, several genomic fragments were subcloned from p1-1812. pHG34B1 has a 2.1-kb insert containing approximately 600 bp of the promoter through the middle of the second exon. pHG34E contains a 9.1-kb fragment containing approximately 2.6 kb of GADD34 promoter and the entire coding region.

Comparison of the human and hamster gadd34 promoters revealed only limited homology. Approximately 250 bp of the proximal promoter showed about 75% homology between species (Fig. 4). However, a TATA box was conserved at −48 and −51 in both human and hamster gadd34, respectively. At −77 (human) and −76 (hamster) a 10-bp binding site for the activator transcription factor/cyclic AMP response element binding family of transcription factors was also conserved. Just distal to this is a GC box, which may bind the Sp1 transcription factor. There is one other GC box in the promoter as well as one in the first intron, although these are not conserved between species (data not shown). In the human promoter, an Alu sequence occurred from −784 to −319.

To roughly map the transcription start site, an antisense genomic riboprobe extending 58 bp more 5’ than the start of the cDNA, Hu34B, was hybridized to mRNA and digested with RNase. The size of the protected fragment was very close to the expected size of 220 bases by comparison with molecular size markers (data not shown).

**DNA Damage Responsiveness of GADD34**—The rodent gadd34 transcript was previously found to be inducible by treatment of cells with various types of DNA damaging agents (16, 32). As expected, the human GADD34 transcript was increased also by these agents in several cell types. In all cells tested, treatment with the alkylating agent MMS led to increased GADD34 mRNA within several hours (Fig. 5). Previous data had shown that maximum transcript levels are seen 4 h following DNA damage in rodent cells (29). In human cells of lymphoid origin, ionizing irradiation caused an increase in GADD34 mRNA levels (Fig. 5, Table I). Similar to BAX, induction of GADD45 by IR plateaued at a low dose (5 Gy), while induction of the p53 regulated GADD45 continued to increase with increasing dose (Fig. 6A). Unlike previously published data for induction of GADD45 by MMS, maximal levels of GADD45 following IR were seen at 8 h but decreased to near basal levels by 12 h (Fig. 6B). This is in contrast to the rodent gadd34 transcript, which was not increased by ionizing radiation in any rodent cell type examined (data not shown). Induction of GADD45 by IR was also not seen in multiple human monolayer cell lines of various tissue origins (Fig. 5, Table I). However, induction by MMS was greater in these adherent cells than in suspension cells (Fig. 5). As a control transcript, levels of GAPDH mRNA varied by less than 2-fold.

To determine whether p53 can directly activate GADD45 expression, as is the case for GADD45, RKo cells were stably transfected with an expression vector encoding E6, a viral protein that targets p53 for turnover and effectively decreases the amount of wild type p53. These cells showed no difference in either the basal level of GADD45 or level after induction by MMS (data not shown). MMS induction of GADD45, for which p53 is required for IR but only contributes for MMS, was decreased with overexpression of E6 (33). GAPDH mRNA was not induc-
 Measurements of 10 specifically hybridized chromosomes 19 showed that GADD34 is located 61% of the distance from the centromere to the telomere of chromosome 19q, an area that corresponds to band 19q13.2. It is interesting to note that this area of chromosome 19 also contains a cluster of known DNA repair genes including ERCC-1, ERCC-2, DNA ligase, and XRCC-1 (35). The apoptosis-associated gene, BAX, is also localized near this region (36).

**DISCUSSION**

GADD34 is a member of a group of genes whose transcript levels are increased following stressful growth arrest conditions and treatment with DNA-damaging agents, hence the designation *gadd* (32). In hamster cells, all five *gadd* genes appear to be coordinately regulated since they all show similar kinetics of induction and spectrums of inducing agents. In most human cells, however, only GADD34, GADD45, and GADD153 are expressed and increased following DNA damage (31). One major difference in expression of the *gadd* genes is that GADD45 induction following IR is strictly dependent on wild type p53. However, induction of GADD34 by IR occurs in certain human cell lines regardless of p53 status. For example, two cell lines with mutant or null p53 show increased GADD34 following IR treatment (Table I). Likewise, not all p53 wild type cells treated with IR exhibit an increase in GADD34 (Table I). For example, in Fig. 5, all cell lines have wild type p53 yet only two show induction of GADD34 by IR. One common property of cells, which showed increased GADD34, is that they all un-

![Image](https://example.com/image.png)

**FIG. 5.** The human GADD34 is inducible by MMS in all cell lines and by ionizing radiation in cell lines that undergo radiation-induced apoptosis. RNase protection assay was done as described under “Experimental Procedures.” RNA was harvested from cells 4 h after initiation of treatment. C, untreated control; M, 100 μg/ml MMS for 4 h; IR, 20 Gy ionizing radiation. ML-1 and WMN are of lymphoid origin and undergo apoptosis following IR. A549 and OVCAR-4 are lung- and ovary-derived monolayers, respectively, and do not undergo appreciable apoptosis following IR. All cell lines are p53 wild type.

**TABLE I**

| Cell line | Cell type | p53 status | \(G_1\) arrest | apoptosis | \(-fold increase in relative mRNA^a\) |
|-----------|-----------|-------------|----------------|-----------|----------------------------------|
| Wild type 53 status with normal p53 function | | | | | |
| ML-1 | Myeloid leukemia | wt/wt | + | + | 8.2 4.7 21.3 |
| WMN | Burkitt’s lymphoma | wt/wt | + | + | 8.5 3.2 7.2 |
| WMN-Bcl2 | Burkitt’s lymphoma | wt/wt | + | + | 3.7 1.4 2.2 |
| AG876 | Burkitt’s lymphoma | wt/wt | + | + | 3.7 2.6 6.4 |
| NL2 | Normal lymphoblastoid | wt/wt | + | + | 6.2 3.4 9.1 |
| FWL | Normal lymphoblastoid | wt/wt | + | + | 8.3 2.8 3.9 |
| MCF-7 | Breast carcinoma | wt/wt | + | + | 1.5 2.0 7.1 |
| AG1522 | Skin fibroblast | wt/wt | + | + | 2.4 1.3 3.7 |
| WI38 | Lung fibroblast | wt/wt | + | + | 1.1 1.4 2.4 |
| RKO | Colorectal carcinoma | wt/wt | + | + | 0.8 0.7 2.8 |
| U2-OS | Osteosarcoma | wt/wt | + | + | 0.7 1.2 2.8 |

**Abnormal p53 status and function**

| Cell line | Cell type | p53 status | \(G_1\) arrest | apoptosis | \(-fold increase in relative mRNA^a\) |
|-----------|-----------|-------------|----------------|-----------|----------------------------------|
| HL-60 | Myeloid leukemia | wt/wt | + | + | 8.3 1.2 1.2 |
| SW480 | Colorectal carcinoma | mut/– | – | + | 3.8 1.0 1.4 |
| CA46 | Burkitt’s lymphoma | mut/– | – | – | 1.0 1.3 0.9 |
| Ramos | Burkitt’s lymphoma | mut/– | – | – | 0.7 1.6 0.7 |
| SG588 | Burkitt’s lymphoma | mut/– | – | – | 0.9 0.5 0.9 |
| MC116 | Burkitt’s lymphoma | mut/– | – | – | 1.2 1.2 1.1 |
| Raji | Lymphoid leukemia | wt/mut | – | – | 1.1 0.8 0.7 |
| H1299 | Large-cell carcinoma | wt/wt | – | – | 0.8 0.8 1.3 |
| KG1a | Myeloid leukemia | wt/mut | – | – | 0.9 0.8 1.0 |
| RKO.m | Colorectal carcinoma | wt/wt, mut | wt/wt, mut | 1.0 1.0 1.5 |
| YA13 | Lung fibroblast | wt/mut | ? | – | 1.2 1.3 1.4 |

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* Human cells with normal or abnormal p53 status were treated with 20 Gy of ionizing radiation. Relative values for samples harvested 4 h after treatment compared with untreated controls were determined by quantitative dot-blot hybridization assay.

* p53 status in various lines is quoted from previous work (5, 26, 42). wt, wild type; mut, mutant.

* Data for \(G_1\) arrest in human cells are taken from Ref. 5 and was carried out at 2 and 4 Gy or at 6 Gy in Burkitt’s lymphoma lines (43).

* Apoptosis in human cells was determined by DNA degradation measured by gel electrophoresis or by filter elution as described previously (26).

* Results for BAX are taken from Ref. 26.

* Results for GADD45 are taken from Refs. 5, 39, and 40.

* Clonal population, transfected with vector overexpressing wild type human BCL2 gene.

* Compared with that of WMN cells, less DNA fragmentation was observed in WMN-Bcl2.

* 48 h after a high dose ionizing radiation, 20 Gray, DNA fragmentation could be detected by gel analysis but it was substantially less than that for ML-1 and the Burkitt’s lines (26).

* No DNA fragmentation in 48 h was observed in fibroblasts strains (44).

* When not determined, the position was left blank.

* Only a single mutant form of p53 was detected. This indicates either that both alleles contained the same mutation or that one allele had been deleted (42).

* Clonal population, transfected with mutant p53 vector, pC53-SCX3 (substitution of alanine for valine at p53 codon 143) (37).

* Lung fibroblast line transformed by SV40.
Fig. 6. Dose response and time course of GADD34 induction by ionizing radiation in ML-1 cells. A, cells were irradiated by the indicated doses, harvested 4 h later, and analyzed by quantitative RNA dot-blot hybridization (see "Experimental Procedures"). B, cells were irradiated with 20 Gy and were harvested at the indicated time after ionizing radiation and analyzed as in A. Data were normalized to the value for unirradiated cells and are shown as percent maximal induction. Results for GADD45 were taken from Refs. 5 and 39–41, and the result for BAX was from Ref. 26.

Fig. 7. Typical metaphase spread showing fluorescence-banded chromosomes. Fluorescent in situ hybridization of chromosome spreads was done as described under "Experimental Procedures." The arrows indicate the positions of the avidin fluorescence signals on chromosomes 19 at band q13.2.

derge apoptosis following this treatment, while cells that do not undergo apoptosis show no increase. Taxol, which can rapidly induce apoptosis regardless of p53 status, does not cause increased GADD34 in any of these cell lines (data not shown). Specifically, in RKO cells, very low concentrations of taxol (1 nM) induce apoptosis yet fail to induce GADD34 (data not shown). Therefore, not all signals for apoptosis cause increased expression of GADD34. Since the transcript is induced by various agents that damage DNA, it is possible that only these types of signals, when they lead to apoptosis, elicit the GADD34 response. On the other hand, treatments such as ultraviolet radiation and methyl methanesulfonate lead to increases in GADD34 in all cells examined yet fail to elicit detectable apoptosis in most cell lines (data not shown). This dual pathway of regulation is reminiscent of GADD45, which requires p53 for regulation of the IR response yet does not require p53 for other DNA damage responses. It is possible that increased Gadd34 may play a role in cellular responses to DNA damage other than apoptosis. Further work will elucidate whether induction of GADD34 following IR is a consequence of apoptosis or if it somehow plays a role in apoptosis.

The regulation of GADD34 by DNA damage and its correlation with apoptosis resembles the regulation of GADD45 in some respects. Both are induced by MMS and UV in all cells but only by IR in some cells. For GADD45, it is known that the IR response is dependent on wild type p53. For GADD34, there may be some regulator which is present only in those cells which undergo apoptosis following IR, perhaps a lymphoid-specific factor. The apoptosis promoting gene, BAX, like GADD34, is also increased following IR induced apoptosis. However, the BAX response is seen only in cells having wild type p53. A reasonable explanation is that the BAX response requires both p53 and the IR/apoptosis proficiency factor required for the GADD34 response.

It is intriguing to note that GADD34 has nearly 50% identity over a 100-amino acid segment with a small herpes simplex virus protein, g134.5, that prevents apoptosis of virally infected cells (18). This area of similarity occurs near the carboxy end of each protein and comprises approximately 40% of g134.5 but less than 15% of GADD34 (Fig. 3). This region of the mouse GADD34 protein (MyD116) can substitute for the equivalent region of g134.5 in suppression of HSV-1-induced apoptosis (20). However, overexpression of GADD34 suppressed cell growth in several human cell lines (16), suggesting that GADD34 has a growth inhibitory effect rather than a survival effect.

It is intriguing that GADD34 was localized to a region of the human genome which harbors several genes which likely are involved in the response to DNA damage. These include four genes involved in the DNA repair process; XRCC-1, ERCC-1, ERCC-2, and Lig-1. The BAX gene, which is a positive effector of apoptosis, a common cellular response to treatments that damage DNA, is also localized just telomeric to this region. Deletions of chromosome bands on 19q have been reported to be associated with gliomas (38). GADD34, as well as the genes mentioned above, maps outside this deletion (37). Previous data have suggested that GADD45 may be involved in a signal transduction pathway controlling cell cycle arrest and/or apoptosis following DNA damage. Specifically, overexpression of GADD34 in human cells leads to both inhibition of cell growth and a change in colony morphology (16). Alterations in such a pathway might contribute to tumorigenesis.

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3 D. Louis, personal communication.
REFERENCES

1. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B., and Craig, R. W. (1991) Cancer Res. 51, 6304–6311
2. Hollander, M. C., and Fornace, A. J., Jr. (1995) in DNA Repair Mechanisms: Impact on Human Diseases and Cancer (Vos, J. M. H., ed) pp. 231–327, R. G. Landes Co., Georgetown, TX
3. Fisher, D. E. (1994) Cell 78, 539–542
4. Dale, M., Nunez, G., Merchant, A. K., Maybaum, J., Rode, C. K., Bloch, C. A., and Castle, V. P. (1994) Cancer Res. 54, 3253–3259
5. 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. (1992) Cell 71, 587–597
6. Agarwal, M. L., Agarwal, A., Taylor, W. R., and Stark, G. R. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8493–8497
7. Cross, S. M., Sanchez, C. A., Morgan, C. A., Schimke, M. K., Ramel, S., Idzerda, R. L., Raskind, W. H., and Reid, B. J. (1995) Science 267, 1353–1356
8. Mellentin, J. D., Murre, C., Donlon, T. A., McCaw, P. S., Smith, S. D., Carroll, A. J., McDonald, M. E., Baltimore, D., and Cleary, M. L. (1989) Science 246, 379–382
9. Clarke, A. R., Purdie, C. A., Harrison, D. J., Morris, R. G., Bird, C. C., Hooper, M. L., and Wyllie, A. H. (1993) Nature 362, 849–852
10. Lowe, S. W., Schmitt, E. M., Smith, S. W., Osborne, B. A., and Jacks, T. (1993) Nature 362, 847–849
11. Wu, X., and Levine, A. J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3602–3606
12. Ron, D., and Habener, J. F. (1992) Genes Dev. 6, 439–453
13. Barone, M. V., Crozat, A. Y., Tabaei, A., Philippson, L., and Ron, D. (1994) Genes Dev. 8, 453–464
14. Smith, M. L., Chen, I., Zhan, Q., Bae, I., Chen, C., Gilmer, T., Kastan, M. B., O'Connor, P. M., and Fornace, A. J. Jr. (1994) Science 266, 1376–1380
15. Carrier, F., Smith, M. L., Bae, I., Kilpatrick, K. E., Lansing, T. J., Chen, C.-Y., Engelstein, M., Friend, S. H., Henner, W. D., Gilmer, T., Kastan, M. B., and Fornace, A. J., Jr. (1994) J. Biol. Chem. 269, 32072–32077
16. Zhan, Q., Lord, K. A., Alamo, I. J., Hollander, M. C., Carrier, F., Ron, D., Kohn, K. W., Hoffman, B., Liebermann, D. A., and Fornace, A. J., Jr. (1994) Mol. Cell. Biol. 14, 2361–2371
17. Lord, K. A., Abdollahi, A., Hoffman, M. L., and Fornace, A. J., Jr. (1990) Cell Growth Differ. 1, 637–645
18. Chou, J., and Roizman, B. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 3286–3270
19. Stewart, N., Hics, G. G., Paraskevakas, F., and Mowat, M. (1985) Oncogene 10, 805–815
20. He, B., Chou, J., Liebermann, D. A., Hoffman, B., and Roizman, B. (1996) Int. J. Oncol. 7, 663–668
21. Devereux, J., Haebeli, P., and Smithies, O. (1984) Nucleic Acids Res. 12, 387–395
22. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
23. McBurney, M. W., Sutherland, I. C., Adra, C. N., Leclair, B., Rudnicki, M. A., and Jardine, K. (1991) Nucleic Acids Res. 19, 5755–5761
24. Walton, M. I., Whyson, D., O'Connor, P. M., Hockenberry, D., Korsmeyer, S. J., and Kohn, K. W. (1993) Cancer Res. 53, 1853–1861
25. Levine, B., Huang, Q., Isaacs, J. T., Reed, J. C., Griffin, D. E., and Hardwick, J. M. (1991) Nature 361, 739–742
26. Zhan, Q., Fan, S., Bae, I., Guillouf, C., Liebermann, D. A., O'Connor, P. M., and Fornace, A. J., Jr. (1994) Oncogene 9, 3743–3751
27. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
28. He, B., Chou, J., Liebermann, D. A., Hoffman, B., and Roizman, B. (1996) Proc. Natl. Acad. Sci. U. S. A. 85, 8800–8804
29. Ron, D., Alamo, I. J., and Habener, M. C. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 51, 849–852
30. Ron, D., Alamo, I. J., and Mitchell, J. B. (1986) Nucleic Acids Res. 14, 5783–5811
31. Lord, K. A., Abdollahi, A., Thomas, S. M., DeMarco, M., Brugge, J. S., Hoffman-Liebermann, B., and Liebermann, D. A. (1991) Mol. Cell. Biol. 11, 4371–4379
32. Fornace, A. J., Jr., Nebert, D. W., Luethy, M. C., Fargnoli, J., and Holbrook, N. J. (1989) Mol. Cell. Biol. 9, 4196–4203
33. Zhan, Q., Fan, S., Smith, M. L., Bae, I., Yu, K., Alamo, I. J., Ron, D., O'Connor, P. M., and Fornace, A. J., Jr. (1996) DNA Cell Biol. 15, 805–815
34. Sylvester, S. L., ap Rhys, C. M., Luethy-Martindale, J. D., and Holbrook, N. J. (1994) J. Biol. Chem. 269, 20119–20125
35. Rubio, M. P., Correa, K. M., Ueki, K., Mihrenweiser, H. W., Gusella, J. F., von, D. A., and Louis, D. N. (1994) Cancer Res. 54, 4760–4763
36. Apte, S. S., Mattei, M. G., and Olsen, B. R. (1995) Genomics 26, 592–594
37. Kuerbitz, S. J., Plunkett, B. S., Walsh, W. V., and Kastan, M. B. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7481–7485
38. von Deimling, A., Louis, D. N., von Ammon, K., Petersen, I., Wiestler, O. D., and Seizinger, B. R. (1992) Cancer Res. 52, 4277–4279
39. Zhan, Q., Fan, S., Bae, I., Kastan, M. B., and Fornace, A. J., Jr. (1994) Cancer Res. 54, 2755–2760
40. Zhan, Q., El-Deiry, W. S., Bae, I., Alamo, I. J., Kastan, M. B., Vogelstein, B., and Fornace, A. J., Jr. (1995) Int. J. Oncol. 6, 937–946
41. Bae, I., Fan, S., Bhatia, K., Kohn, K. W., Fornace, A. J., Jr., and O'Connor, P. M. (1995) Cancer Res. 55, 2387–2393
42. O'Connor, P. M., Jackman, J., Jondle, D., Bhatia, K., Magrath, I., and Kohn, K. W. (1993) Cancer Res. 53, 4776–4780
43. Fan, S., El-Deiry, W. S., Bae, I., Freeman, J., Jondle, D., Bhatia, K., Fornace, A. J., Jr., Magrath, I., Kohn, K. W., and O'Connor, P. M. (1994) Cancer Res. 54, 5824–5830
44. Fornace, A. J., Jr., Nagasawa, H., and Little, J. B. (1988) Mutat. Res. 70, 323–336
