Dexamethasone-induced Gene 2 (dig2) Is a Novel Pro-survival Stress Gene Induced Rapidly by Diverse Apoptotic Signals*

Zhengqi Wang, Michael H. Malone, Michael J. Thomenius, Fei Zhong, Fang Xu, and Clark W. Distelhorst†

From the Departments of Medicine and Pharmacology, Comprehensive Cancer Center, Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, Ohio 44106

Glucocorticoid hormones induce apoptosis in lymphoid cells. This process requires de novo RNA/protein synthesis. Here we report the identification and cloning of a novel dexamethasone-induced gene designated dig2. Using Affymetrix oligonucleotide microarray analysis of ~10,000 genes and expressed sequence tags, we found that the expression of dig2 mRNA is significantly induced not only in the murine T cell lymphoma lines S49.A2 and WEHI7.2 but also in normal mouse thymocytes following dexamethasone treatment. This result was confirmed by Northern blot analysis. The induction of dig2 mRNA by dexamethasone appears to be mediated through the glucocorticoid receptor as it is blocked by RU486, a glucocorticoid receptor antagonist. Furthermore, we demonstrated that dig2 is a novel stress response gene, as its mRNA is induced in response to a variety of cellular stressors including thapsigargin, tunicamycin, and heat shock. In addition, the levels of dig2 mRNA were up-regulated after treatment with the apoptosis-inducing chemotherapeutic drug etoposide. Though the function of dig2 is unknown, dig2 appears to have a pro-survival function, as overexpression of dig2 reduces the sensitivity of WEHI7.2 cells to dexamethasone-induced apoptosis.

Glucocorticoid hormones are potent inhibitors of T cell proliferation and inducers of thymocyte death (1, 2). Hence, glucocorticoids are frequently used as immunosuppressives to treat a broad range of autoimmune and inflammatory disorders and prevent graft rejection following bone marrow or organ transplantation. Also, glucocorticoids are effective agents for treatment of lymphomas and lymphoid leukemias, including acute lymphoblastic leukemia and chronic lymphocytic leukemia (3–5).

Glucocorticoids suppress lymphocyte proliferation and survival by two fundamental processes. First, glucocorticoids arrest proliferating lymphocytes in the G1 phase of the cell cycle (6). Second, glucocorticoids induce apoptosis in immature lymphocytes (7). The negative effects of glucocorticoids on lymphocyte proliferation and survival are mediated through the glucocorticoid receptor, a ligand-activated transcription factor that induces or represses transcription of individual genes and gene networks (8). The transactivation activity of the glucocorticoid receptor appears essential for glucocorticoid-induced cell death, although glucocorticoid-induced “death genes” have not yet been identified (reviewed in Ref. 2).

Recent developments in DNA microarray technology permit a large number of cellular transcripts to be analyzed in parallel fashion (9). Using this technology, we have analyzed gene expression profiles of dexamethasone (Dex)1-treated lymphocytes to identify genes that are potentially involved in mediating or regulating glucocorticoid-induced apoptosis. For this purpose, we have utilized three separate but related Dex-sensitive model systems, i.e. the S49.A2 murine T cell lymphoma line, the WEHI7.2 murine T cell lymphoma line, and primary murine thymocytes. Although there were many genes whose expression was either induced or repressed by Dex in these model systems, only seven genes or expressed sequence tags (ESTs) were induced or repressed by Dex in all three systems. One of the ESTs was particularly intriguing, because it was induced very rapidly. In this report we show that this EST represents a novel stress response gene that is induced in response to Dex and a variety of other cellular stressors. Although the function of the protein encoded by this gene remains unknown, its overexpression inhibits apoptosis induction by Dex, suggesting that it has a pro-survival activity.

EXPERIMENTAL PROCEDURES

Cell Culture—S49.A2 and WEHI7.2 murine T-cell lymphoma lines were gifts of Drs. Diane Dowd and Roger Miesfeld, respectively. Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Bio-Whittaker) supplemented with 2 mM glutamine and 10% (v/v) heat-inactivated bovine calf serum at 37 °C in a 7% CO2 atmosphere under high humidity. Dex was purchased from Sigma, and a stock solution was prepared in 100% ethanol. S49.A2 and WEHI7.2 cells were seeded at 1 × 105 cells/ml for 12 h before treatment with 1 μM Dex for 6, 12, 18, and 24 h. The control cells at each time point were treated with ethanol vehicle only. Cell viability was monitored by trypan blue dye staining.

Thymus Isolation—C57BL/6J female mice at 8–11 weeks old (Jackson Laboratory) were sacrificed by CO2 asphyxiation. Thymus glands were removed, rinsed in ice-cold growth medium (tMEM supplemented with 10% fetal bovine serum, 2 mM l-glutamine, 12.5 units/ml penicillin, and 12.5 μg/ml streptomycin), and then dispersed through a steel wire mesh into 5 ml of fresh, cold growth medium per thymus. The suspension of thymocytes was filtered through a tube with a cell strainer cap (BD Discovery Labware) to remove connective tissue. For

Received for publication, April 9, 2003
Published, JBC Papers in Press, May 7, 2003, DOI 10.1074/jbc.M303723200

© 2003 by The American Society for Biochemistry and Molecular Biology, Inc.

This paper is available online at http://www.jbc.org Printed in U.S.A.
each experiment, thymocytes were pooled from five mice, diluted to 2–3 × 10⁶ cells/ml in warm growth medium, treated with 1 μM Dex or ethanol vehicle control, and grown in a humidified 7% CO₂ atmosphere at 37 °C. For the microarray experiment, thymus glands were isolated from adrenalecctomized female mice.

**Oligonucleotide Array Expression Analysis**—Gene expression analysis was performed essentially as described in the Affymetrix GeneChip expression analysis technical manual. Total RNA was harvested from both Dex-treated (1 μM) and vehicle control populations at each time point by Trizol (Invitrogen) extraction. Trizol cell lysates were separated into aqueous and organic phases by the addition of chloroform to a final concentration of 20% (v/v). The aqueous phase was purified and concentrated using an RNaseasy minicolumn (Qiagen). DNA complementary to total RNA samples were reverse transcribed using SuperScript reverse transcriptase (Invitrogen) and a T7-(dT)₂₄ primer (Operon). This cDNA was used as a template for the synthesis of biotinylated cRNA using the T7 MEGAScript kit from Ambion. Biotinylated cRNA probes were fragmented and hybridized to MG-U74A(v2) GeneChips (Affymetrix) using an Affymetrix GeneChip Fluidics Station 400 and standard Affymetrix protocols. Fluorescence intensities were captured with a GeneArray Scanner (Hewlett-Packard).

GeneChip image files were processed using Microarray Analysis Suite, version 5.0 (Affymetrix). Probe cells displaying irregular fluorescence intensity over the area of the cell were excluded from subsequent analyses. To facilitate comparison between samples and experiments, the trimmed mean signal of each array was scaled to a target intensity of 1500. Comparative analysis between treatment and control samples for each time point was performed with the Affymetrix statistical algorithm using default parameters. To compensate for gene expression changes occurring in the control cultures over time, each treated sample was compared with a control sample that was split and harvested in parallel with the treated population. Metric files from expression and comparison analyses were exported to Microsoft Access XP for further analysis. In this work, genes called "absent" and signal ≥500 in treatment or control samples for inductions or repressions, respectively) and were determined by the statistical algorithm to be changed 2-fold or greater (change call "+" or no change) and signal ≥500 in treatment or control samples for inductions or repressions, respectively. To increase stringency, genes meeting the above criteria were filtered further to include only those that also were changed in the same direction (change call "+" or change) in at least one adjacent time point regardless of magnitude.

**cDNA Cloning of dig2**—Reverse transcription-PCR was performed using 5'-CTCCTGTGGCCCTTATCTC-3' and 5'-CTCAAGTGACATGGCTATAC-3' as primers with first strand DNA as templates that were reverse transcribed using total RNA purified from S49.A2 and normal mouse thymocytes. PCR products were cloned into the pGEM-T easy vector (Promega), and DNA inserts were sequenced in both directions by Cleveland Genomics Inc.

**Northern Blot Analysis**—Total RNA was extracted from cultured cells using the Trizol reagent (Invitrogen) followed by purification through an RNaseasy minicolumn (Qiagen). Total RNA (10 μg for the sample from cultured cells and 2.5 μg for the sample from primary thymocytes) was separated in a 1.0% agarose-formaldehyde gel and transferred to a GeneScreen Plus membrane (PerkinElmer Life Sciences) in 10× SSC (contents of 1× SSC, 0.15 M NaCl, and 15 mM sodium citrate, pH7.0) by capillary blotting. The RNA was fixed to the membrane by cross-linking with UV (245 nm, 30 s, 1200 μJ) using a Stratalinker (Stratagene). Membranes were hybridized with a 32P-labeled probe in QuikHyb (Stratagene) at 65 °C. Membranes were subsequently washed at 65 °C twice for 15 min each in 2× SSC, once for 30 min in 1× SSC, once for 5 min in 0.1× SSC, and once briefly in ethanol. Membranes were exposed to X-ray film with intensifying screens (Eastman). Membranes were then stripped and rehybridized with a 32P-labeled probe in a 1× SSC mixture for 2 h at 65 °C.

**Data Analysis**—The intensity of AI849939 expression with or without 1 μM Dex for the indicated hours. Data shown are graphical representations of the hybridization signal intensity of AI849939 expression with or without 1 μM Dex for the indicated hours.
Fig. 2. Nucleotide sequence of mouse dig2 cDNA and its expression in multiple tissues. A, the predicted amino acid sequence for dig2 is shown by the single-letter code under the nucleotide sequence. The numbers of amino acid positions relative to the initiating Met are shown in parentheses under those of the nucleotide positions. The underlined sequence (three nucleotides or one amino acid) for Dig2 is deleted to make the short form of Dig2 (GenBank accession number AY260552). B, distribution of dig2 in early developmental stages and various mouse tissues. A master blot membrane was purchased from Clontech (catalog no. 7771-1) and contains normalized loading of poly(A) RNA from 22 different mouse tissues and seven different control RNAs and DNAs.

Fig. 3. Both actinomycin D and RU486 inhibited Dex-induced dig2 mRNA expression. A, actinomycin D (Act D) inhibited but cycloheximide (CHX) did not block Dex-induced dig2 mRNA expression. S49.A2 cells were maintained in the presence of ethanol (control), 1 μM Dex, 1 μg/ml actinomycin D or 10 μg/ml cycloheximide, as indicated. B, RU486, a glucocorticoid receptor antagonist, prevented the induction of dig2 mRNA by Dex. S49.A2 cells were treated with ethanol (control), 1 μM Dex, or 50 μg/ml of RU486, as indicated. 10 μg of total RNA per lane was used for Northern blot analysis. The staining of 28 S ribosomal RNA was used as a loading control.

Fig. 4. Dex induced the stress response genes herp and gadd153 but did not induce grp78 and grp94 genes in S49.A2 cells. Total RNA from S49.A2 cells that were left untreated (ethanol, control) or treated by 1 μM Dex for the indicated hours were analyzed. Each lane contained 10 μg of total RNA. The staining of 28 S ribosomal RNA was used as a loading control.
The staining of 28 S ribosomal RNA was used as a loading control.

Stripped and probed for dig2 (for dexamethasone-induced gene 2). dig2 is transcribed as a single mRNA species around 1.8 kb. To clone the coding region of dig2, reverse transcription-PCR was carried out using RNA purified from Dex-treated S49.A2 and normal mouse thymus gland. The sequence of the PCR products revealed that dig2 has two isoforms with only three nucleotides (one amino acid) difference between them (Fig. 2A). Northern blot analysis using a mouse master blot (Clontech) revealed that dig2 is ubiquitously expressed not only in multiple mouse tissues in the adult but also in early developmental stages from embryo days 7 to 17 (Fig. 2B).

**RESULTS**

**Cloning of a Novel Dex-Induced Gene**—In response to Dex, S49.A2 and WEHI7.2 cells undergo cell growth arrest first, followed by apoptosis. In contrast, primary murine thymocytes are already growth-arrested and rapidly undergo apoptosis following Dex treatment (1, 2). Because all three systems undergo apoptosis and because apoptotic pathways are generally highly conserved, we sought to identify Dex-induced genes common to all three cell systems. To identify Dex-regulated gene(s), we utilized oligonucleotide microarray technology. Total RNA was purified from S49.A2 and WEHI7.2 cells treated with or without 1 μM Dex for 6, 12, 18, and 24 h or isolated from primary thymocytes treated with 1 μM Dex for 2 h. The control cells at each time point were treated with ethanol vehicle only. Isolated RNA was then hybridized to Affymetrix MG-U74A(v2) oligonucleotide microarrays representing ~10,000 genes and ESTs. Pairwise analysis (comparing gene expression between Dex-treated and time-matched untreated control cells) was used to identify differentially expressed genes. The expression of a total of 132 genes in S49.A2, 296 genes in WEHI7.2, and 59 genes in primary thymocytes were changed significantly (10). However, only seven genes were coordinately regulated by Dex in S49.A2, WEHI7.2, and the primary thymocytes. The fold induction for these seven genes after Dex treatment at each time point is listed in Table I. One of the ESTs, AI849939, which was induced 2.3–5.7-fold after Dex treatment in S49.A2, WEHI7.2, and the primary thymocytes, was selected for further study.

The results of the microarray for AI849939 as presented in hybridization signal intensity are shown in Fig. 1A. This was further confirmed by Northern blot analysis of RNA from Dex-treated S49.A2 cells, WEHI7.2 cells, and primary thymocytes using AI849939 as a probe (Fig. 1B). As shown in Fig. 1C, AI849939 was rapidly induced as early as 30 min after the addition of Dex to S49.A2 cells. Furthermore, AI849939 was also up-regulated when WEHI7.2 cells were treated with doses of Dex as low as 5 nM (data not shown). A BLAST search of the public data base revealed that AI849939 has 100% sequence homology with a previously uncharacterized gene, RIKEN cDNA (AK017926), and its single open reading frame codes for a putative protein of 229 amino acids. Therefore, we have designated it dig2 (for dexamethasone-induced gene 2). dig2 is transcribed as a single mRNA species around 1.8 kb. To clone the coding region of dig2, reverse transcription-PCR was carried out using RNA purified from Dex-treated S49.A2 and normal mouse thymus gland. The sequence of the PCR products revealed that dig2 has two isoforms with only three nucleotides (one amino acid) difference between them (Fig. 2A). Northern blot analysis using a mouse master blot (Clontech) revealed that dig2 is ubiquitously expressed not only in multiple mouse tissues in the adult but also in early developmental stages from embryo days 7 to 17 (Fig. 2B).

**Dex-induced dig2 Expression Requires Transcriptional Activation Mediated through the Glucocorticoid Receptor**—Because Dex rapidly induced dig2 mRNA expression, we questioned whether this induction is transcriptionally regulated. As shown in Fig. 3A, actinomycin D (Act D) treatment inhibited dig2 mRNA induction by Dex, whereas cycloheximide (CHX) did not. This result suggests that the induction of dig2 by Dex is transcriptionally controlled. It is also notable that dig2 seems to have a relatively short half-life, because the abundance of dig2 mRNA decreased to nearly undetectable levels from 3 to 12 h after actinomycin D treatment. RU486, a glucocorticoid...
receptor antagonist, prevented the induction of dig2 mRNA by Dex in S49.A2 cells (Fig. 3B). Furthermore, dig2 was not induced by Dex in S49.143R cells, which contain defective glucocorticoid receptors (data not shown). This indicates that the induction of dig2 mRNA expression requires a functional glucocorticoid receptor.

dig2 Is a Novel Stress Response Gene—Among the seven genes regulated by Dex (Table I), herp has been reported as an endoplasmic reticulum (ER) stress response gene induced by homocysteine, β-mercaptoethanol, tunicamycin (TU), A23187, and thapsigargin (TG) (11). Therefore, we checked whether Dex treatment is able to induce an ER stress response. Though the treatment with Dex induced the expression of herp and gadd153/chtac1 in S49.A2 cells as confirmed by Northern blot analysis, treatment with Dex did not induce the expression of the typical ER stress response genes encoding the glucose-regulated proteins (Grps), grp78 and grp94 (Fig. 4). Furthermore, the ER stress inducers, TG and TU, did cause a rapid induction of dig2 in S49.A2 cells (Fig. 5A). The induction of dig2 expression by TG and TU was not dependent on a functional glucocorticoid receptor, because dig2 mRNA was induced dramatically in S49.143R cells, which do not have functional glucocorticoid receptors (Fig. 5B). This up-regulation of dig2 by TG and TU was not limited to T lymphoma cells, as it was also induced in MDA-MB-468 cells, a human breast cancer cell line (Fig. 5C). These results indicated that dig2 is a novel stress response gene. The Grps and heat shock proteins (Hsps) represent the two major groups of stress-response proteins. In Fig. 6A, we have shown that a brief heat shock treatment (43 °C, 5 min) also up-regulates dig2 gene expression in S49.A2 cells. Furthermore, when S49.A2 cells were treated with 10 mM β-mercaptoethanol (Fig. 6B) or cultured under conditions of osmotic stress (pMEM with 0.15 M NaCl, 5 min) (Fig. 6C), the expression of dig2 mRNA was also induced, although the level of induction is not as significant as that following TU and TG treatment. These findings further support the concept that dig2 is a stress response gene.

Expression of the dig2 Gene in Response to Other Apoptotic Inducers—The preceding stress inducers also mediate apoptosis. Therefore, we tested whether the induction of dig2 expression is associated generally with apoptosis. As revealed by Northern hybridization, when S49.A2 cells were treated with 250 nM staurosporine (STS), a protein kinase C inhibitor, dig2 expression was not induced (Fig. 7A). However, etoposide, another apoptosis inducer in S49.A2 cells, induced dig2 expression significantly (Fig. 7B). dig2 induction by Dex, TG, TU, and etoposide was not affected by Bcl-2 overexpression (Fig. 7C).

Overexpression of Dig2 Desensitizes Cells to Dex-induced Apoptosis—To assess the effect of Dig2 on the viability of WEHI7.2 cells, we cloned the cDNA encoding Myc-tagged Dig2 into the expression vector, pIRESneo2 (Clontech), in both sense and antisense orientations. We obtained clones stably expressing Myc-tagged Dig2 when WEHI7.2 cells were transfected with the sense orientation vector. Though the Dig2 protein has an estimated molecular mass of 25 kDa, Western analysis revealed that Myc-tagged Dig2 gives rise to a protein product with a size estimated around 35 kDa (Fig. 8A). This suggests that Dig2 is post-translationally modified. No clones were obtained when WEHI7.2 cells were transfected with the antisense vector. This finding suggests that the function of Dig2 may be essential for cell survival. Consistent with this conclusion, we found a lower percentage of dead cells in untreated cultures of WEHI7.2 cells that stably express Myc-tagged Dig2, versus control cells transfected with the empty vector (5.3 ± 1.9% (at 24 h) and 4.3 ± 2.8% (at 36 h) versus 13.5 ± 6.1% (at 24 h) and 13.8 ± 5.2% (at 36 h), n = 4, p < 0.05 (at 24 h) and <0.02 (at 36 h), respectively) (Fig. 8B). To determine whether Dig2 regulates cell death induction by Dex, cells stably expressing Myc-tagged Dig2 and empty vector control cells were treated with 1 μM Dex for 24 and 36 h. The percentage of dead cells following Dex treatment was much less in cells expressing Myc-tagged Dig2 compared with cells transfected with empty vector (9.6 ± 0.9% (24 h after Dex) and 40.7 ± 3.9% (36 h after Dex) versus 28.7 ± 8.5% (24 h after Dex) and 73.0 ± 6.5% (36 h after Dex), n = 4, p < 0.02 (24 h) and <0.0001 (36 h), respectively) (Fig. 8B). We have examined two independently selected clones of Neo and Dig2 and obtained essentially the same results in independent clones (data not shown). These findings indicate that Dig2 has a pro-survival function.
We report the identification and cloning of a novel stress response gene, dig2. This gene has widespread tissue distribution. Moreover, it is strongly induced in both lymphoid and non-lymphoid cells by TG, TU, and etoposide, as well as being moderately induced by cellular stressors including osmotic stress, β-mercaptoethanol, and heat shock. Also, dig2 transcription is induced by Dex only in lymphoid cells (Fig. 1), whereas it is not induced by Dex in non-lymphoid MDA-MB-468 cells (data not shown). dig2 is a mouse homologue of a novel hypoxia-inducible factor 1-responsive gene, rtp801, reported during the course of the present work (12). Also, we detect an EST corresponding to dig2 among a list of p53-regulated transcripts (13). Collectively, these findings indicate that dig2 is induced in response to a wide variety of cellular stressors.

One remarkable observation about dig2 is the rapidity of its induction. dig2 induction by thapsigargin was much more rapid than the induction of the well characterized ER stress response genes grp78 and grp94 (Fig. 5). This is much more rapid than most of the other Dex-induced transcripts detected with microarrays. Furthermore, although the induction of dig2 by Dex was mediated through the glucocorticoid receptor and blocked by the glucocorticoid receptor antagonist RU486, the mechanism of dig2 induction is presently uncertain. Moreover, the increase in levels of dig2 mRNA following Dex treatment was blocked by actinomycin D but not by cycloheximide. These findings suggest that transcriptional up-regulation was at least in part responsible for dig2 induction and that new protein synthesis (e.g. of an intermediate signaling factor) was not required. Together, these findings, including the rapidity of induction, suggest the possibility of direct transactivation of the dig2 gene by the glucocorticoid receptor. However, analysis of the 5′ dig2 promoter sequence failed to detect a consensus sequence corresponding to a typical glucocorticoid response element. Moreover, an 800 base pair region 5′ to the transcription initiation site of dig2 was cloned into a luciferase reporter plasmid. However, Dex failed to induce luciferase activity when this reporter plasmid was expressed by transient transfection in WEHI7.2 cells (data not shown). Thus, based on these preliminary findings, there is no evidence of direct regulation of a dig2 promoter/enhancer region by the glucocorticoid receptor.

Further work will be needed to uncover the mechanism of dig2 induction by Dex and other cellular stressors.

The mammalian stress response is an evolutionarily conserved mechanism that allows cells to respond to adverse environmental or metabolic conditions. A wide range of stresses, including heat shock, inhibition of energy metabolism, and oxidative stress induce expression of Hsps (14). Hsps assist in the recovery from stress either by degrading or repairing damaged proteins (protein refolding). The term “glucose-regulated proteins” encompasses a variety of ER chaperones that can be induced by glucose starvation or depletion of ER calcium (15). Perturbations in ER function disrupt protein folding, leading to accumulation of unfolded proteins and protein aggregates that are detrimental to cell function and survival. Hence, the induction of Grps is referred to as the unfolded protein response. A number of different proteins are induced by ER stress. Many of these reside in the ER lumen (e.g. Grp78 and Grp94) or are located on the ER transmembrane (e.g. Herp), but several are in non-ER locations, including gadd153/CHOP (nucleus), GLUT-1 (plasma membrane) and asparagine synthetase (cytoplasm) (15, 16).

We are interested in investigating the mechanism of apoptosis induction by glucocorticosteroid hormones. Exposure of lymphocytes to Dex induces a unique form of cell stress. Dex quickly up-regulates the ER stress gene herp and also induces the expression of gadd153/chop (Fig. 4), an ER stress and apoptosis-related protein. Here, we have found that Dex treatment rapidly induces a stress response gene, dig2. However, Dex does not elevate the expression of grp78 and grp94. This suggests that the induction of dig2 by Dex is mediated through a signaling pathway different from that mediating induction of grp78 and grp94.

The events of cell stress and cell death are linked, such that the molecular chaperones induced in response to stress appear to function at key regulatory points in the control of apoptosis (14). Hsp expression is modulated by many conditions that lead to apoptosis. Exposure of cells to stress activates a survival response via the induction of Hsps. Moreover, induction of Grps is essential for maintenance of cell survival following ER stress (15). Here, the induction of dig2 expression may well have been a survival response when cells were exposed to the apoptosis inducer Dex. This hypothesis is supported by the evidence that overexpression of Dig2 did inhibit Dex-induced apoptosis (Fig. 8). Moreover, enforced expression of rtp801, a homologue of dig2, inhibited hypoxia-induced apoptosis, although, paradoxically, this same gene appeared to promote apoptosis when expressed in non-dividing cells (12). Thus, although the mechanism of dig2/rtp801 action is unknown, this gene appears to play a role in regulating cell survival.

In summary, the present work identifies dig2 as a novel stress response gene that is rapidly induced in response to a variety of cellular stresses. The induction of dig2 may serve a protective function, delaying or inhibiting apoptosis. Its mechanism of action and potential other functions remain to be elucidated. Moreover, the signal transduction pathway(s) that mediate dig2 induction in response to such a wide range of cellular stresses provides a challenge for further investigation.

Acknowledgments—We thank Dr. Nikki Holbrook for providing the gadd153 cDNA and Dr. Amy Lee for giving us the cDNAs for dig2 expression may well have been a survival response when cells were exposed to the apoptosis inducer Dex. This hypothesis is supported by the evidence that overexpression of Dig2 did inhibit Dex-induced apoptosis (Fig. 8). Moreover, enforced expression of rtp801, a homologue of dig2, inhibited hypoxia-induced apoptosis, although, paradoxically, this same gene appeared to promote apoptosis when expressed in non-dividing cells (12). Thus, although the mechanism of dig2/rtp801 action is unknown, this gene appears to play a role in regulating cell survival.

REFERENCES
1. Ashwell, J. D., Lu, F. W., and Vaccio, M. S. (2000) Annu. Rev. Immunol. 18, 309–345
2. Distelhorst, C. W. (2002) Cell Death Differ. 9, 6–19
3. Forbes, I. J., Zalewski, P. D., Giannakis, C., and Cowled, P. A. (1992) Exp. Cell Res. 196, 367–372
4. Gaynor, P. S., and Carrel, A. L. (1999) Adv. Exp. Med. Biol. 457, 593–605
5. Smets, I. A., Salomons, G., and van den Berg, J. (1999) Adv. Exp. Med. Biol. 457, 607–614
6. Harrison, J. M., Norman, M. R., Pfolkes, B. J., and Thompson, E. B. (1979) J. Cell Biol. 89, 267–278
7. Wylie, A. H. (1980) Nature 284, 555–556
8. Yamamoto, K. R. (1985) Annu. Rev. Genet. 19, 209–252
9. Staudt, L. M., and Brown, P. O. (2000) Annu. Rev. Immunol. 18, 829–859
10. Wang, Z., Malone, M. H., He, H., McColl, K. S., and Distelhorst, C. W. (April 2, 2003) J. Biol. Chem. DOI 10.1074/jbc.M301843200
11. Kokame, K., Agarwala, K. L., Kato, H., and Miyata, T. (2000) J. Biol. Chem. 275, 32846–32853
12. Shoshani, T., Faerman, A., Mett, I., Zelin, E., Tenne, T., Gorodin, S., Moshel, Y., Elbaz, S., Budanov, A., Chajut, A., Kalinski, H., Kamer, I., Rezen, A., Mor, O., Keshet, E., Leshkovitz, D., Einat, P., Skalitzer, R., and Feinstein, E. (2002) Mol. Cell. Biol. 22, 2283–2293
13. Kraman, K., Kaminski, N., Rechavi, G., Jakob-Hirsch, J., Amariglio, N., and Givol, D. (2001) Oncogene 20, 3449–3455
14. Jolly, C., and Morimoto, R. I. (2000) J. Natl. Cancer Inst. 92, 1564–1572
15. Lee, A. S. (2001) Nat. Rev. Mol. Cell Biol. 3, 411–421
Dexamethasone-induced Gene 2 (dig2) Is a Novel Pro-survival Stress Gene Induced Rapidly by Diverse Apoptotic Signals
Zhengqi Wang, Michael H. Malone, Michael J. Thomenius, Fei Zhong, Fang Xu and Clark W. Distelhorst

J. Biol. Chem. 2003, 278:27053-27058.
doi: 10.1074/jbc.M303723200 originally published online May 7, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M303723200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 15 references, 2 of which can be accessed free at
http://www.jbc.org/content/278/29/27053.full.html#ref-list-1