

Low-Dose Irradiation Alters the Transcript Profiles of Human Lymphoblastoid Cells Including Genes Associated with Cytogenetic Radioadaptive Response

Matthew A. Coleman,^a Eric Yin,^a Leif E. Peterson,^b David Nelson,^a Karen Sorensen,^a
James D. Tucker^{a,1} and Andrew J. Wyrobek^{a,2}

^a *Biology & Biotechnology Research Program, Lawrence Livermore, National Laboratory, Livermore, California 94551; and*

^b *Department of Medicine, Baylor College of Medicine, Houston, Texas 77030*

Coleman, M. A., Yin, E., Peterson, L. E., Nelson, D., Sorensen, K., Tucker, J. D. and Wyrobek, A. J. Low-Dose Irradiation Alters the Transcript Profiles of Human Lymphoblastoid Cells Including Genes Associated with Cytogenetic Radioadaptive Response. *Radiat. Res.* 164, 369–382 (2005).

Low-dose ionizing radiation alters the gene expression profiles of mammalian cells, yet there is little understanding of the underlying cellular mechanisms responsible for these changes or of their consequences for genomic stability. We investigated the cytogenetic adaptive response of human lymphoblastoid cell lines exposed to 5 cGy (priming dose) followed by 2 Gy (challenge dose) compared to cells that received a single 2-Gy dose to (a) determine how the priming dose influences subsequent gene transcript expression in reproducibly adapting and non-adapting cell lines, and (b) identify gene transcripts that are associated with reductions in the magnitude of chromosomal damage after the challenge dose. The transcript profiles were evaluated using oligonucleotide arrays and RNA obtained 4 h after the challenge dose. A set of 145 genes (false discovery rate = 5%) with transcripts that were affected by the 5-cGy priming dose fell into two categories: (a) a set of common genes that were similarly modulated by the 5-cGy priming dose irrespective of whether the cells subsequently adapted or not and (b) genes with differential transcription in accordance with the cell lines that showed either adaptive or non-adaptive outcomes. The common priming-dose response genes showed up-regulation for protein synthesis genes and down-regulation of metabolic and signal transduction genes (>10-fold differences). The genes associated with subsequent adaptive and non-adaptive outcomes involved DNA repair, stress response, cell cycle control and apoptosis. Our findings support the importance of TP53-related functions in the control of the low-dose cytogenetic radioadaptive response and suggest that certain low-dose-induced alterations in cellular functions are predictive for the risk of subsequent genomic damage. © 2005 by Radiation Research Society

INTRODUCTION

Exposure to low doses of ionizing radiation (<10 cGy) alters gene expression profiles in cells and animal tissues (1–3) but, under certain circumstances, protects cells against the damaging effects of subsequent higher-dose exposures (4, 5). This protective phenomenon, generally known as the adaptive response, has been broadly observed in mammalian systems (6–9) and can reduce cytogenetic damage, enhance survival, increase resistance to infection, and reduce tumor incidence (9–16).

It has been established that low-dose irradiation alters the expression of genes associated with diverse cellular functions (3, 17–20) and different forms of ionizing radiation show qualitative differences in the pathways affected (i.e. γ and β -particle radiation) (21). There have been several studies of gene transcript expression in cells exposed to radiation (1–3, 18, 22–25), yet only two have assessed the global cellular effects of low doses (<10 cGy) (1, 22), and none have used gene transcript profiling to investigate the mechanisms of adaptive response.

The adaptive response phenotype has been associated with DNA damage repair and stress response functions based on functional and single-gene investigations. Exogenous endonucleases that generate DNA breaks have induced adaptive response, suggesting that DNA damage is involved (4), and inhibitors of protein synthesis can block adaptive response, suggesting that adaptive response requires *de novo* protein synthesis (26, 27). Inhibitors of the DNA repair-related protein poly(ADP-ribose) polymerase (PARP) can block adaptive response, further implicating repair processes (28–30). DNA-PK, ATM and TP53, which are involved in DNA damage recognition and signaling, have also been implicated in adaptive response (31). It has

¹ Current address: Department of Biological Sciences, Wayne State University, Detroit, MI 48202.

² Address for correspondence: Biomedical Sciences Division, L-448, Bioscience Directorate, 7000 East Avenue, Livermore, CA 94551; e-mail: wyrobek1@llnl.gov.

been suggested that TP53 plays a major role in adaptive response through a p38MAPK signaling pathway along with other effectors that may include BRCA1, BRCA2, IRF1, RB, ERK1/2 and JNK/SAPK (32, 33). The DNA repair protein DIR1 has been implicated in adaptive response by increasing the rate of repair (34, 35), and APE1, a base excision repair endonuclease, may be involved in adaptive response by linking repair to oxidative pathways (36). Although there have been numerous studies of individual genes and their proteins, there has been no genome-scale assessment of the responses of cells to low-dose radiation or of the gene expression associations with the adaptive response phenomenon.

Three hypotheses were tested in our study: (a) Exposure of cells to acute low-dose radiation (priming dose) prior to an acute high-dose exposure (challenge dose) induces changes in the transcriptome profiles that persist beyond the challenge dose. (b) Specific gene transcript changes induced by the priming dose are independent of whether a cell line will show adaptive response or not, while (c) transcript changes in other genes will be predictive of adaptive response outcomes. We previously characterized numerous human lymphoblastoid cell lines for cytogenetic adaptive response phenotypes by micronucleus analyses (13) and selected three lines for the current study that were reproducibly adapting or non-adapting after a 5-cGy priming dose in biological replicate experiments. Our study design used oligonucleotide microarrays containing ~12,000 human genes. The RNA sampling time (i.e. 4 h after the challenge dose) was selected to allow us to compare our new gene transcript findings with literature reports of corresponding protein changes that may occur within the same time window after the challenge dose (11, 33).

METHODS

Our experiments used cells of three human lymphoblastoid cell lines (GM15036, GM15510 and GM15268) that we had previously characterized for their cytogenetic radioadaptive responses in biological replicate analyses of micronucleus frequencies (13). Briefly, cells in logarithmic growth in suspension cultures were exposed to sham radiation or a 5-cGy priming dose, followed 6 h later by 2 Gy (challenge dose), and then analyzed ~20 h later for relative effects of the priming dose compared to sham irradiation on the micronucleus frequencies. Aliquots of cells were frozen at multiple times after the challenge dose. Our study focused on samples collected 4 h after the 2-Gy challenge dose from cultures that had previously been treated with or without the 5-cGy priming dose.

Cell Culture

The lymphoblastoid cell lines were obtained from the Coriell Cell Repositories at American Tissue Culture Collection (Manassas, VA). Use of these publicly available cell lines was deemed exempt from institutional IRB approval. Cells were suspension grown in RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 15% fetal calf serum (Sigma-Aldrich, Chicago, IL) containing antibiotic-antimycotic mixture [100 U/ml penicillin G sodium, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B as Fungizone® in 0.85% saline (Invitrogen), and 2 mM L-glutamine (Invitrogen)]. All cultures were grown in a humidified 95%

air/5% CO₂ atmosphere at 37°C and maintained at a concentration of 1 to 10 × 10⁵ cells/ml.

Irradiations and RNA Preparation

A total of 1 × 10⁷ cells for each cell line were collected and irradiated using a ¹³⁷Cs Mark 1 Irradiator (J. L. Shepherd and Associates, Glendale, CA) with a priming dose of 5 cGy followed 6 h later with a challenging dose of 2 Gy. The negative control was sham-irradiated (neither priming nor challenge dose) and the positive control received only the challenge dose. Dose rates were 0.3 and 0.6 Gy/min for the priming and challenge doses, respectively. After irradiation, cells were grown for an additional 4 h at 37°C and then harvested by centrifugation, resuspended in approximately 250 µl of medium and frozen at -80°C. Total RNA was extracted using the TRIZOL protocol (Invitrogen). RNA was treated with RNase-free DNase to remove any contaminating genomic DNA (BD Biosciences Clontech, Palo Alto, CA), and RNA quality was confirmed by agarose gel electrophoresis with ethidium bromide staining or using an Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA). Purified total RNA was stored at -80°C.

Oligonucleotide Microarrays

Isolated RNA was converted to double-stranded cDNA following the Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA). The Enzo BioArray HighYield Transcript Kit was then used for RNA amplification-labeling (Enzo Biochem, New York). The Affymetrix HU95A gene chips were hybridized using 10 µg of the fragmented complementary RNA followed by processing in an Affymetrix Fluidics Workstation (Affymetrix). Hybridization signals were detected through the use of an argon-ion laser scanner (Agilent Technologies), and output for pixel intensities and confidence calls for each of the genes detected on the array were generated with Affymetrix Microarray Suite 5 (MAS-5) software.

Statistical Analysis

Eighteen Affymetrix HU95A chips were used for this study: two experimental replicate arrays for each of the nine combinations of three cell lines and three treatments. *P* values and log₂-transformed intensities were obtained from Affymetrix's MAS-5 software and normalized in two steps. First, the pair of chips for each replicate was normalized using Astrand's quantile normalization method (37) to produce chips with the same overall intensity distribution. Second, the normalized intensities across the 18 chips were adjusted by a chip-specific factor to ensure that the median intensity of the 12,626 genes on each chip was identical across the 18 chips.

MAS-5 *P* values were used to determine genes with a positive signal. The *P* values for each chip were adjusted by the Benjamini-Hochberg method to control the per-chip false discovery rate. The "mt.rawp2adjp" procedure in Bioconductor (38) was used to perform the adjustment. Only genes with a false discovery rate-adjusted *P* value not exceeding 0.01 were selected for subsequent analysis as described below.

The per-chip expression data corresponding to genes with a positive signal were combined in a two-step process to obtain an initial analytical data set consisting of 4,768 genes. The first step consisted of producing three separate cell line data sets. Each cell line data set consisted of expression data for all the genes for which a signal was detected in at least one of the six chips for that cell line. The second step consisted of combining the three cell line data sets into an initial analytical data set. The analytical data set consisted of genes present in any of the three cell line data sets. Thus each gene in the analytical data set could have expression data for one, two or three cell lines and might be expressed in one, two or three of the treatment conditions assayed in each cell line.

Genes with differential expression across the nine treatment combinations were detected by means of an *F* test. A separate *F* test was performed on each gene. Each *F* test evaluated differences among three, six or nine treatments according to whether data were present for one,

two or three cell lines. The P values for these 4,768 genes were adjusted using the same Benjamini-Hochberg procedure described above. A total of 1,775 genes had an adjusted F test P value <0.05 , of which there were 1,208 present in all cell lines. These 1,208 genes were selected for further analysis to detect differences in response to radiation exposure across the cell lines. In this context, the “priming dose response” for a cell line is defined to be the difference between the response to a 2-Gy exposure with and without a preceding 5-cGy exposure. This difference was evaluated using an F test for interaction between cell line and the two treatments. Only those responses detected in two or more cell lines were examined. The resulting P values were again adjusted to control for false discovery rate, and 520 genes with an adjusted P value not exceeding 0.20 were selected for further analysis.

For gene annotation and functional classification we used EASE (<http://www.david.gov/EASE>) and MAPPFinder [Gladstone Institute at UCSF, <http://www.genmapp.org/> (39)], which use the “Gene Ontology” (GO) consortium [<http://www.geneontology.org/> (40)] database for the analysis of pathways and gene relationships. We applied a ratio criterion of greater than 1.8 for (5 cGy + 2 Gy)/2 Gy signals for the 520 genes using MAPPFinder to search for GO annotations associated with the significant differential transcripts affected by the priming dose. This level of cutoff was based on prior criteria established for microarray data in our laboratory (1, 41) and is consistent with the literature. The standardized difference scores were used to rank GO categories based on the relative number of gene expression changes within each GO map. An assigned standardized difference score [z score (37)] greater than 2 was used as a measure of effect both for the priming dose and for association with the micronucleus radioadaptive end point. The z score and micronucleus measure of the adaptive response were then used to select candidate genes with ontologies and pathway information.

The analysis tool CLUSTER and Factor Analysis Using Varimax Orthogonal Rotation [CLUSFAVOR; <http://mbcr.bcm.tmc.edu/genepi> (42)] was used to identify associations between both priming dose and radioadaptation transcript responses of the 520 genes using their level of change (5 cGy + 2 Gy/2 Gy). Cluster analysis was based on Euclidean distance without standardization to identify the natural grouping of gene expression profiles (42, 43). The color gradient was based on the rankings of the expression changer for all genes in all cell lines.

Semi-quantitative PCR

Confirmation of transcript levels for several associated radioadaptive modulated genes was performed by RT-PCR of cDNA produced from mRNA from each of the three cell lines. RNA from samples used for microarray analyses was reverse transcribed using Superscript II reverse transcriptase (Life Technologies, Rockville, MD) and an oligo-dT primer. PCR was performed in 100 μ l reactions, using the Platinum[®] RT-PCR ThermoScript[™] One-Step System (Invitrogen). The *GAPD* gene, which did not show a change in expression (data not shown), was used as an internal control. The primers selected were as follows: *GAPD* (200 bp) Forward primer: GTCTAGAAAAACCTGCCAAA, reverse primer: ATACCAGGAAATGAGCTTGA; *ATM* (406 bp) forward primer: ACCAGAGATATTGTGGATGG, reverse primer: TTGAGATTTTTGGGGTCTATG; *P125 Phospholipase* (399 bp) forward primer: TCCAGATTTGGACCTAAAAG, reverse primer: CTCTGAAGAGCGAAAAGGTA; *MYC* (410 bp) forward primer: TGAGGAGGAACAAGAAGATG, reverse primer: TGAGGAGGAACAAGAAGATG; *IFNR2* (380 bp) forward primer: CAGTTGGAACCTTTGAGTGG, reverse primer ATATAACCA TCCCAAGGTC; *HSP8A* (404 bp) forward primer: GGAAGACATTG AACGTATGG, reverse primer AATCAACCTCTTCAATGGTG; and *CBF2* (403 bp) forward primer: GCTCTGGAAGGATGATATG, reverse primer GATCCCATATTTTCATCAA. PCR conditions were optimized to be performed as follows for all transcripts: 25–30 cycles at 94°C for 15 s; 52°C for 30 s; 72°C for 1 min, followed by 1 cycle at 72°C for 10 min.

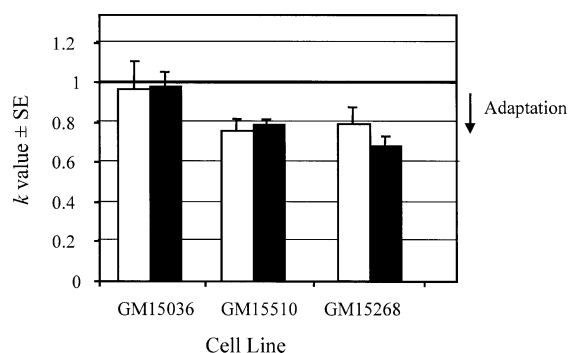


FIG. 1. Selecting cytogenetically adapted and non-adapted human lymphoblastoid cell lines. Micronucleus analysis was used to quantify the radioadaptive response for three cell lines (13). The k value is the ratio of micronucleus frequencies in cells that received the priming dose as well as the challenge dose (5 cGy + 2 Gy) compared to cells that received only the challenge dose (2 Gy). The k values of less than one indicate that adaptation occurred, while values equal to or close to one show a lack of adaptation. The white and black bars represent the results of the first and second independent biological replicate experiments, respectively.

RESULTS

Selection of Genes Associated with Low-Dose Priming Effects

We investigated the gene transcript profiles of three lymphoblastoid cell lines (Fig. 1) that were previously characterized for their cytogenetic adaptive response by micronucleus analysis (13). Cell lines GM15268 and GM15510 were reproducibly adaptive, showing 20–30% reductions in the frequency of micronuclei in cells that received the 5-cGy priming dose followed by a 2-Gy challenge dose. In contrast, line GM15036 was reproducibly non-adapted in replicate experiments, showing no detectable change in micronucleus frequencies associated with the 5-cGy priming dose. RNA was isolated for gene transcript analyses from three experimental groups for each cell line: 4 h after sham irradiation (0 cGy), 4 h after a dose of 2 Gy that was preceded by a sham priming dose (2 Gy), and 4 h after a challenge dose of 2 Gy that was preceded by a 5-cGy priming dose (5 cGy + 2 Gy).

Statistical analyses identified a set of genes whose gene transcript levels were differentially modulated in cells after they had received the 5-cGy priming dose followed by the challenge dose relative to cells that received only the 2-Gy challenge dose. The hybridization signals across ~12,000 oligonucleotide probe sets, i.e. genes, showed little variability between replicate chips (correlation coefficient >0.98). There was detectable signal in at least one experimental group across the three cell lines for 4,768 genes (false discovery rate 1%). Differential gene expression among the three groups identified 1,775 genes (F ratio, false discovery rate 5%) of which 1,208 genes had detectable gene transcript signals in all three cell lines for both the 2-Gy and 5 cGy + 2-Gy samples. A subset of 520 genes showed differential responses (>1.8 -fold) between the 2-

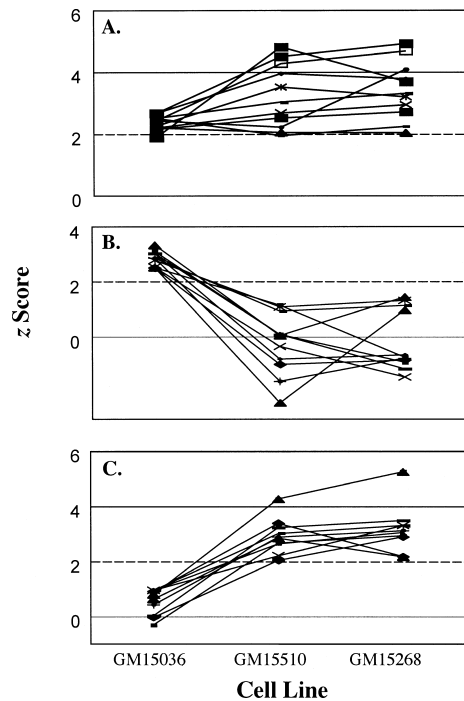


FIG. 2. Gene ontology (GO) maps associated with the common 5-cGy priming dose effects, radioadaptation or non-adaptation. Thirty-six GO maps were associated with priming dose effects. MAPPFinder was used for comparing significant differential gene expression either associated with the priming dose (panel A) or associated with the radioadaptive outcomes (panels B and C). Shown are the top 12 maps with the largest z scores for the number of genes with observed expression differences relative to the number of affected genes expected. Panel A: Human GO categories associated with a high z score for genes across all three cell lines irrespective of adaptive response outcome were Hs_endoplasmic reticulum, Hs_intracellular 10, Hs_sphingolipid metabolism, Hs_microsome, Hs_cell 10, Hs_integral plasma membrane protein, Hs_cell 41, Hs_cell 5, Hs_intracellular 30, and Hs_plasma membrane 3. Panel B: GO categories associated with a high z score for genes in the two radioadaptive cell lines relative to unchanged or lower for the non-adaptive cell line included Hs_response to biotic stimulus, Hs_transmembrane receptor, Hs_signal transduction 4, Hs_intracellular 31, Hs_cytoplasm 9, Hs_chemokine, Hs_cytokine, Hs_cell-cell signaling, Hs_response to wounding 2, Hs_cell 21, Hs_cell 9, and Hs_membrane fraction 9. Panel C: GO categories associated with high z score for genes in the non-radioadaptive cell line relative to unchanged or lower across the two radioadaptive cell lines included Hs_transcription regulation 7, Hs_enzyme 11, Hs_intracellular 29, Hs_ligand binding or carrier 12, Hs_cell adhesion molecule, Hs amino acid activation, Hs_tRNA ligase, Hs_membrane fraction 4, Hs_lyase, Hs_ubiquitin C-terminal hydrolase, Hs_enzyme 22, and Hs_cytochrome c oxidase.

Gy and 5 cGy + 2-Gy groups in at least one cell line (false discovery rate 20%; 145 genes with false discovery rate 5%; see supplemental data at microarray.llnl.gov for a complete listing of genes). The 520 genes fell into 713 Gene Ontology (GO) categories with at least one gene per category (see supplemental data at microarray.llnl.gov). The top five GO categories accounted for 40% of the genes: protein biosynthesis (~10%, 54 genes), DNA-dependent transcription (8%, 43 genes), ATP binding activity (~8%, 41 genes), immune response (~7%, 38 genes), and regulation of tran-

TABLE 1
Genes that are Up-Regulated in Response to the Priming Dose, Independent of the Adaptive Response Outcome (Group 1)^a

Gene	Accession number	Relative expression			Average ^b
		GM15036	GM15510	GM15268	
<i>EEF1A1</i>	J04617	>10	>10	10	10.0
<i>RPS2</i>	X17206	>10	>10	9	9.7
<i>RPL28</i>	U14969	>10	3.4	4.8	6.1
<i>RPS15A</i>	W52024	10	1.7	4.7	5.5
<i>RPL8</i>	Z28407	7.4	2.3	6.5	5.4
<i>RB1</i>	NM_000321	3.4	2.6	>10	5.3
<i>RPLP1</i>	M17886	8.3	3.2	4.3	5.3
<i>RPS4X</i>	M58458	9.7	1.8	4.1	5.2
<i>DRAP1</i>	U41843	>10	3.3	2.1	5.1
<i>RPS11</i>	X06617	9	2.7	3	4.9
<i>RPS10</i>	U14972	7.1	2.8	4.2	4.7
<i>RPL6</i>	X69391	7.6	2.6	3.5	4.6
<i>RPS20</i>	L06498	8	2	3.5	4.5
<i>GNB2L1</i>	M24194	9.2	1.7	2.5	4.5
<i>RPS3</i>	X55715	6.1	3	4.1	4.4
<i>RPL18A</i>	X80822	6.9	3.8	2.5	4.4
<i>RPL1</i>	M64241	5.6	3.2	4.3	4.4
<i>RPL14</i>	D87735	8.3	2.5	2.2	4.3
<i>RPS8</i>	X67247	6.7	2.5	3.4	4.2
<i>RPLP0</i>	M17885	4.7	2.8	5.1	4.2

^a See supplemental material at microarray.llnl.gov for complete listing.

^b Average relative expression among three cell lines.

scription (~7%, 35 genes). Reducing the size of the input list to 145 genes by lowering the false discovery rate to 5% did not significantly alter the distribution of the top GO categories (data not shown).

The GO categories with differential z scores (Fig. 2) and cluster analyses (Fig. 3) identified four groups of genes whose transcription was modulated by the 5-cGy priming dose. Genes of groups 1 and 2 (Tables 1 and 2) were modulated in the same direction among all cell lines (up or down, respectively) when the challenge dose was preceded by the priming dose, and the responses were independent of their adaptive response. We consider these transcripts to be a measure of the effects of the 5-cGy priming dose. Grouping by functional category for the priming dose genes of Groups 1 and 2 are shown in Table 3, columns one and two. Group 3 genes (Table 4) were also modulated by the priming dose but showed higher expression levels after the priming dose in the non-adapted cell line than in the adapted cells lines. Group 4 genes (Table 5) were also modulated by the priming dose but showed relatively higher expression in the adapted cell lines than in the non-adapted cell line. The Group 3 and 4 transcripts were therefore indicative of radioadaptation. Grouping by functional category for the radioadaptive genes of Groups 3 and 4 are shown in Table 3, columns three and four.

Genes Associated with Priming-Dose Effects, but Independent of Adaptive Response Outcome

Figure 2A illustrates the top 12 GO maps with consistently elevated z scores (>2) across all three cell lines,

TABLE 2
Genes that are Down-regulated in Response to the Priming Dose, Independent of the Adaptive Response outcome (Group 2)^a

Gene	Accession number	Relative expression			
		GM15036	GM15510	GM15268	Average ^b
<i>SPTLC1</i>	Y08685	0.1	0.2	0.1	0.1
<i>ATP2B1</i>	J04027	0.1	0.2	0.1	0.1
<i>KIAA0004</i>	D13629	0.1	0.1	0.3	0.2
<i>H-SP1</i>	X68194	0.1	0.2	0.2	0.2
<i>GLDC</i>	D90239	0.1	0.3	0.1	0.2
<i>TNFRSF8</i>	M83554	0.2	0.3	0.1	0.2
<i>ZMPSTE24</i>	Y13834	0.1	0.2	0.3	0.2
<i>W28612</i>	W28612	0.3	0.1	0.2	0.2
<i>TFRC</i>	X01060	0.3	0.1	0.2	0.2
<i>TFRC</i>	M11507	0.3	0.1	0.2	0.2
<i>RZF</i>	AF037204	0.3	0.1	0.2	0.2
<i>LAMP2</i>	U36336	0	0.2	0.4	0.2
<i>CD19</i>	M28170	0	0.2	0.4	0.2
<i>SLC9A6</i>	AF030409	0.2	0.3	0.2	0.2
<i>SLC7A5</i>	M80244	0.5	0.1	0.1	0.2
<i>HMGCR</i>	M11058	0.2	0.2	0.3	0.2
<i>SQLE</i>	D78130	0.4	0.2	0.1	0.2
<i>ITGB1</i>	X07979	0.1	0.2	0.4	0.2
<i>TMP21</i>	L40397	0.3	0.2	0.3	0.3
<i>SLC2A5</i>	M55531	0.3	0.2	0.3	0.3

^a See supplemental material at microarray.llnl.gov for complete listing.

^b Average relative expression among three cell lines.

which identifies cellular pathways involved in the common transcriptional responses across the three cell lines, independent of their adaptive responses. Cluster analyses (Fig. 3) identified two clusters of genes that were similarly modulated by the priming dose across all three cell lines, independently of their adaptive response: “all up” (Fig. 3, group 1) and “all down” (Fig. 3, group 2). There was a very large (~100-fold) range of responses between groups 1 and 2 (Tables 1 and 2, and supplemental data at microarray.llnl.gov). The top 20 genes of Group 1 showed 4–10-fold higher gene transcript levels in cells that received the priming dose prior to the challenge dose compared to cells that received the challenge dose alone (Table 1). The top 20 genes of group 2 (Table 2) showed 5–10-fold reductions in gene transcript levels.

Protein synthesis was the major cellular function associated with the common up-regulated genes of Group 1 (Table 3). Strikingly, 48 of 60 Group 1 genes (Fig. 3) involve ribosomal functions and protein biosynthesis. These genes included the elongation factor *EEF1A1* (~11-fold elevation), which is involved in joining aminoacyl-tRNAs to the ribosomes and various structural protein components of ribosomes (e.g. *RPL28*, *RPS2* and *RPS15A*). Several cell cycle and signal transduction genes were also identified as up-regulated, including *GNB2L1* (commonly known as *RACK*) and the tumor suppressor gene, *RBI*.

In contrast, the common down-regulated group 2 genes (Table 3) were dominated by metabolism functions (e.g. *GLDC*, *LAMP2* and *KIAA0004*). Other group 2 genes en-

coded multiple membrane-bound proteins such as ion transporters and proteins involved in cell adhesion-related functions (e.g. *ATP2B1*, *CD19*, *CD44*, *CD53* and the transferring receptor *TFRC* and the sodium-hydrogen exchanger *SLC96A*). Two group 2 genes were directly associated with cell cycle control and DNA repair pathways (*WEE1* and *PRKARIA*/protein kinase, cAMP-dependent).

Gene Transcripts Associated with Differential Adaptive Response Outcomes (Adaptive and Non-adaptive)

The 520-gene set was also analyzed to identify priming-dose-affected genes whose transcript levels were differentially associated with either adaptive or non-adaptive outcomes. The hybridization signals for the two cell lines that reproducibly radioadapted (GM15510 and GM15268) were more highly correlated with each other (correlation coefficient = 0.75) than with the non-adapted cell line (GM15036) (0.55 and 0.56, respectively), suggesting that there were global transcription changes associated with two possible outcomes: adaptive or non-adaptive.

The GO categories (Fig. 2B and C) and hierarchical clustering (Fig. 3) identified two groups of genes associated with adaptive response outcomes (group 3 in Tables 4 and group 4 in Table 5; also see supplemental material at microarray.llnl.gov). Tables 4 and 5 list the top 20 genes in groups 3 and 4, respectively, and illustrate the large (>100-fold) range of differences between the responses in these two groups.

The genes of groups 3 and 4 represented diverse cellular functions associated with adaptive response outcomes (Table 3). Group 3 (i.e. genes with lower transcript levels in adapting cell lines) includes genes associated with cell cycle/proliferation and signal transduction (e.g. *MYC*, *STAT1*, *BTG1*, *CCNI* and *GNB1*); apoptosis-related genes (e.g. *TNF*, *CASP8* and *NFKB1*); ubiquitin-dependent protein degradation genes (e.g. *E2EPF*, *EDD*, *KIAA0317*, *PSMA6*, *UBE21*, *USP6* and *USP9X*); translational control genes involving amino acid activation and tRNA ligation (e.g. *GARS*, *WARS* and *YARS*); protein modification genes involved specifically in stress response (*PRDX4* and *GADD45A*); DNA double-strand break repair (i.e. *PRKDCIP* and *XRCC7*); and oxidoreductases genes related to general cellular stress responses (e.g. *TXNRD1*, *IDH2*, *MTRR*, *PDIA3* and *PDIA6*).

Group 4 (i.e. genes with higher expression in adapting cell lines) includes genes involved in DNA repair (e.g. *ATM*, *SP100* and *ERCC5/XPG*); cell cycle control and signal transduction (e.g. *CETN3*, *MPHOSPH10*, *P125* and *CREM*, *EBNA1BP2* and *LPXN*); and stress response (e.g. *PRDX1*, *HSPA8/HSP70* and *HSPD1/HSP60*). Group 4 also included six functionally uncharacterized and/or non-annotated genes (i.e. *FRG1*, *RES4-25*, *DKFZP564*, *F0522*, *MEP50*, *NME1* and *CBF2*).

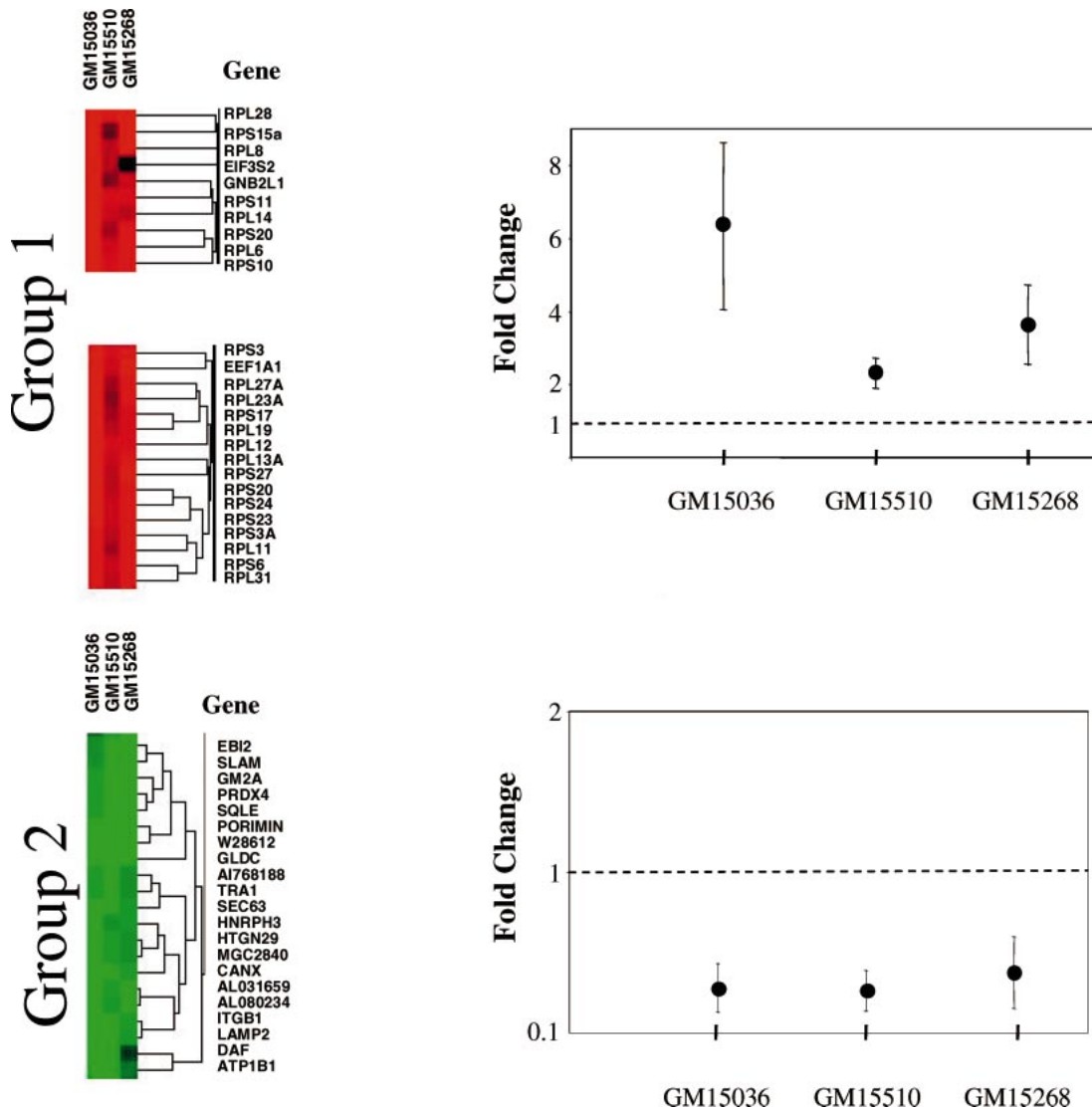


FIG. 3. Cluster analyses of genes associated with the common 5-cGy priming dose effects, radioadaptation and non-adaptation. Four expression profiles were identified using Euclidean distance-based hierarchical clustering. Gene clusters and heat maps of their change are shown for comparisons between the 2-Gy and 5 cGy + 2-Gy experimental groups (see Materials and Methods). For each cluster of genes, the relative expression and standard deviations are plotted for each cell line. The dashed line represents a relative expression of 1 (i.e. no change). Group 1 represents genes that were up-regulated more than twofold in all three cell lines. Group 2 are genes that were down-regulated by more than twofold in all three cell lines. Groups 3 and 4 represent those genes that were differentially modulated between the non-adapted cell line and the two adapted cell lines, GM15268 and GM15510. Group 3 represents those genes with larger change in the non-adapted cell line, while group 4 contains genes with larger changes in the two adapted cell lines.

Adaptive Response-Associated Genes that were Linked to TP53 Functions

Several group 3 and 4 genes that discriminated between adaptive and non-adaptive outcomes encode proteins that have been associated with TP53-related functions. As shown in Fig. 4, the cell lines that had an adaptive response up-regulated groups of genes associated with DNA repair and stress response while down-regulating genes associated with cell cycle control and apoptosis compared to the results for the line that did not adapt after the priming-challenge dosing regimen. The microarray findings for key

genes of Fig. 4 were validated by RT-PCR (Fig. 5) based on their position on the left and right side of the adaptive response map shown in Fig. 4, for example DNA repair and stress responses (*ATM*) and cellular proliferation (*MYC*). As shown in Fig. 4, DNA damage sensing was implicated by increased transcript levels in adapted lines for phosphatidylinositol kinases (i.e. *ATM* and *YWHAQ*/14-3-3 family of proteins) which are known to be involved in multiple signaling interactions with mitogen-activated kinases such as MAPKp38 and CHK2, which are themselves capable of forming protein-protein interactions with TP53.

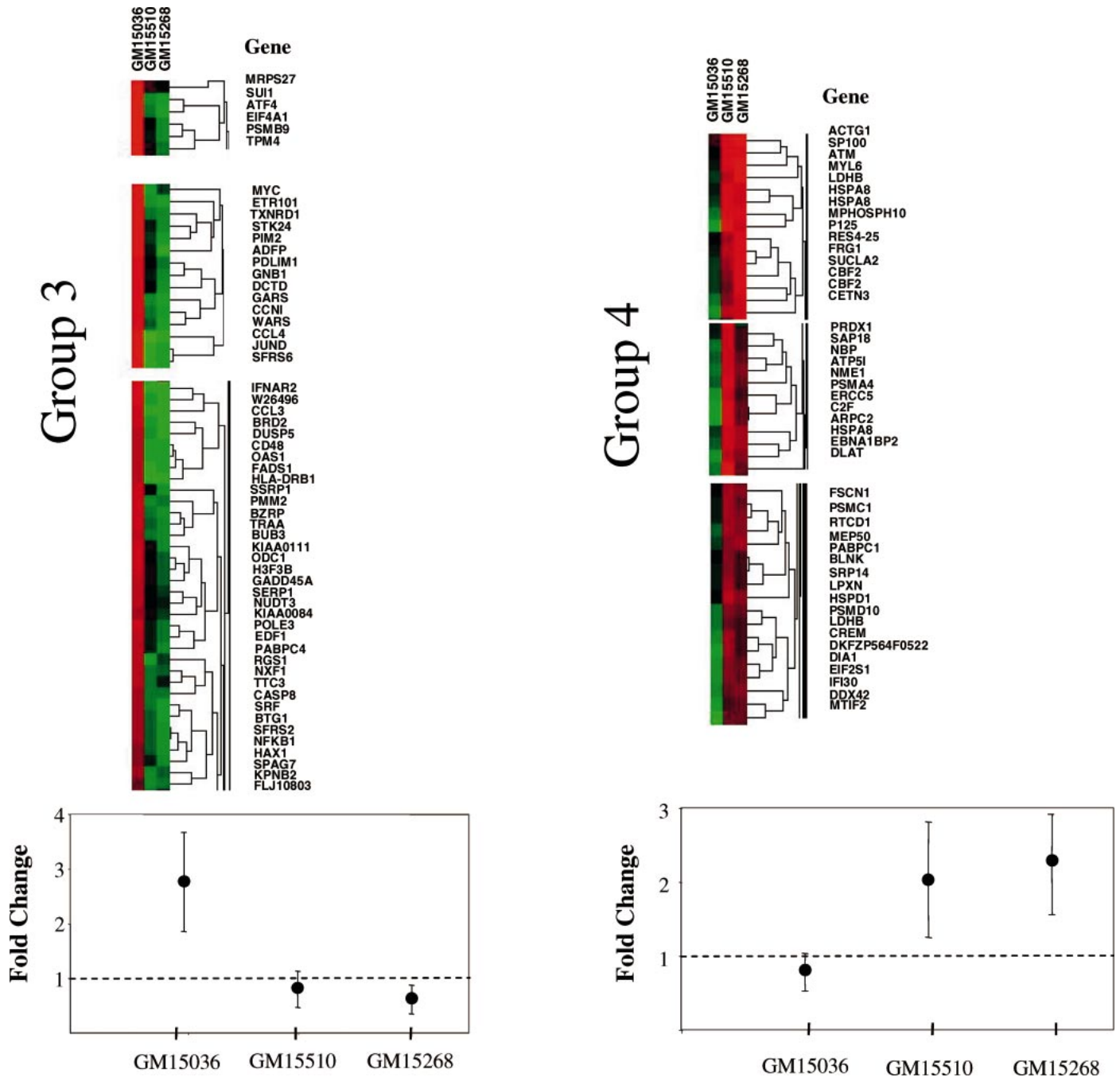


FIG. 3. Continued.

Expression levels of *ATM* increased more than twofold in both adapted lines, with no change in the non-adapted line, as confirmed by RT-PCR (Fig. 5). In addition, both *P125*, a cell cycle-related transcript, and *CBF2*, a CAAT transcription factor, showed a greater than twofold increase on the microarray, and the differential expression was confirmed by RT-PCR. The involvement of nuclear foci in adaptation was implicated by transcript increases in the *SP100* gene that interacts with PML, a possible regulator of TP53 function. DNA repair was implicated by increases in *ERCC5* transcripts in the lines that adapted. HSP70 family

members [*HSPA8* (HSP70) and *HSPD1* (HSP60)] were also up-regulated in the cell lines that adapted, which can be related to a role in downstream protein degradation. The *HSPA8* microarray transcript findings were confirmed by RT-PCR.

Several genes with lower expression in the adapted lines suggested another set of TP53 functional links to cellular proliferation and apoptosis (e.g. *CASP8*, *JUND*, *MYC*, *NFKB*, *SSRP1*, *TNF* and *IFNAR2*). The *MYC* and *IFNAR2* transcript microarray finding was verified by RT-PCR (Fig. 5). Interestingly, these transcripts showed in-

TABLE 3
Pathway Analysis of Genes Associated with Priming Dose or Radioadaptive Effects

Pathway Response group	Priming dose genes		Radioadaptive genes	
	Group 1, common "up" response after priming dose	Group 2, common "down" response after priming dose	Group 3, differential response, lower in adapting cell lines	Group 4, differential response, higher in adapting cell lines
Apoptosis		<i>CD53, PORIMIN, TNFRSF10B</i>	<i>CASP8, DAD1, HAX1, NFKB1, TNF, TNFRSF17</i>	
Cell adhesion		<i>CD58, ENTPD1, ITGB1, KIAA0911</i>	<i>CD44, CD164</i>	<i>ICAM2</i>
Cell cycle	<i>RBI</i>	<i>WEE1</i>	<i>CCNI, BTGI, BUB3, EDF1, EMP3, MYC, PIM2, SRF, PRKDCIP</i>	<i>CETN3, MPHOSPH10, P125</i>
Chemokine			<i>CCL3, CCL4, SCYA5, SCYA22, SCYA3, SCYA4</i>	<i>CRM1</i>
DNA repair		<i>PRKDC</i>	<i>PRKARIA</i>	<i>ATM, ERCC5, SP100, NBP</i>
Immune response			<i>CD48, IFITM1, HLA-DMA, HLA-DMB, TCRA</i>	<i>BLNK, NKTR</i>
Metabolism	<i>ODC1, RPS13</i>	<i>ARL6IP, CD9, CYP1B1, EBP, ENTPD1, GGH, GLDC, GM2A, GUSB, HMGCR, KIAA0004, KIAA0088, LAMP1, LAMP2, PPT1, SLC2A5, SLC9A6, SPTLC1, TFRC, ZMPSTE24</i>	<i>AHCY, ADA, IDH2, IDH3G, ODC1, PMM2, SC5DL, SIAT1</i>	<i>DLAT, FDFIT1, LDHB, OS9, SUCLA2</i>
Protein degradation		<i>KIAA0317, RPN2, RZF, TL132, USP9X</i>	<i>CTSC, EDD, PSEN1, SPAG7, UBE21</i>	<i>IFI30, PSMA4, PSMC1, PSMC6, PSMD10, USP6</i>
Protein biosynthesis	<i>RPL (5, 6, 8–12, 13A, 14, 15, 17, 19, 18A, 21, 23, 23A, 24, 27A, 28–32, 34, 38), RPLP0, RPLP1, RPS (2, 3, 3A, 4X, 5–11, 14, 15A, 17, 19, 20, 23, 24, 27), EEF1A1, EEF1G</i>	<i>FMR1, CANX, MRPS6</i>	<i>EIF3S5, EIF4A1, E2EPF</i>	
RNA metabolism		<i>DDX3, HNRPH3, RNP24</i>	<i>DDX21, NXF1, PABPC1, SFRS2, SFRS6</i>	<i>DDX42, RTCD1, PABPC1, RTCD1</i>
Signal transduction	<i>DRES9, GNB2L1, TEBP</i>	<i>ADAM10, AKAP1, ATP1B3, ASAH, CD19, CD59, CNIH, CR2, EBI2, EPB72, SLAM, SORL1, TNFRSF8</i>	<i>AMFR, BZRP, GNB1, IFNAR2, NUDT3, PRKCB1, RGS1, STAT1, STAT3, SSRP1, STK24, VEGFB, YWHAZ</i>	<i>CREM, CTNNB1, EBNA1, BP2, LPXN, PFTK1, YWHAQ, TTK</i>
Stress response	<i>HADH2</i>	<i>HSPA5, PDIA3, PDIA6, PRDX4, SQLE</i>	<i>HERPUDI, GADD45A, MTRR, LITAF, SERP1, TXNRD1</i>	<i>DIA1, HSPA8, HSPD1, IF130</i>
Transcription	<i>DRAP1, NSEPI</i>	<i>H-SP1, TRAI, CHD1</i>	<i>ATF5, EIF4A2, ETR101, JUND, SMARCA4, TCEA1</i>	<i>CBF2, DEK, SMARCA2, TCF12, ZNF148</i>
Translation			<i>GARS, SARS, WARS, YARS</i>	<i>EIF2S1, MTIF2, NARS, RARS</i>

creased levels in the non-adapted cell line when compared to the adapted lines, suggesting a role in the adaptive response for modulation of genes combined on apoptosis. Transcript levels for several transcription factors

controlled by *NFKB* induction such as *STAT1* and *STAT3* that control cell proliferation were also lower in the radioadaptive cell lines. The *GADD45A* stress response gene was up-regulated in the non-adapted line, suggest-

TABLE 4
Genes with Relatively Larger Changes
in Expression when the Cell does not Adapt
(Group 3)

Gene	Accession number	Relative expression			Ratio of effect ^a
		GM15036	GM15510	GM15268	
<i>SCYA4</i>	J04130	3.3	0.03	0.03	110.0
<i>SCYA3</i>	D90144	2.5	0.2	0.1	16.7
<i>ATF5</i>	AB021663	7.8	0.7	0.6	12.0
<i>IFNAR2</i>	L42243	2.7	0.3	0.2	10.8
<i>JUND</i>	X56681	3.2	0.3	0.4	9.1
<i>SFRS6</i>	AL031681	3.2	0.3	0.4	9.1
<i>SARS</i>	X91257	8.2	1	0.8	9.1
<i>ETR101</i>	M62831	3.7	0.6	0.4	7.4
<i>LITAF</i>	AF010312	6.5	1.2	0.6	7.2
<i>EIF4A1</i>	D13748	4.8	1.1	0.6	5.6
<i>GARS</i>	U09510	3.1	0.7	0.4	5.6
<i>MYC</i>	V00568	3.9	0.5	0.9	5.6
<i>TM9SF2</i>	U81006	0.8	0.2	0.1	5.3
<i>CD48</i>	M37766	2.1	0.4	0.4	5.3
<i>OAS1</i>	X04371	2.1	0.4	0.4	5.3
<i>ADFP</i>	X97324	3.4	1	0.3	5.2
<i>PIM2</i>	U77735	3.6	0.9	0.5	5.1
<i>NP</i>	X00737	6.7	1.5	1.2	5.0
<i>PRKDC1P</i>	U85611	5.8	1.4	1	4.8
<i>WARS</i>	X59892	2.9	0.8	0.4	4.8

^a Calculated ratio of relative expression between non-adaptive and adaptive cell lines by averaging the results for the two adaptive cell lines.

ing differential DNA damage responses for adaptive and non-adaptive outcomes.

DISCUSSION

Our gene transcript study demonstrates that exposure of lymphoblastoid cell lines to low-dose radiation (5 cGy) followed 6 h later with a high-dose exposure (2 Gy) altered the transcription profiles of a large number of genes as measured 4 h after the 2-Gy exposure. The gene transcript responses fell into two broad categories, (1) those with common responses across all three cell lines, independent of their adaptive response outcomes, and (2) those with differential responses associated with their adaptive response outcomes. Thus our study is a simultaneous investigation of expression changes that persist after low-dose exposure, as well as expression changes that may be predictive of the likelihood of subsequent cytogenetic damage. In contrast, previous studies of the effects of low-dose ionizing radiation on transcript levels have been limited to the effects of single acute exposures (1, 22, 44) or repeated high-dose exposures for measuring outcomes (45, 46). Our study is also the first genome-wide inspection of transcript profiles associated with adaptive response outcomes.

Table 3 contrasts the cellular functions of genes associated with the common priming dose responses compared to those associated with the adaptive response outcomes. There was a range of more than 100-fold in responses among the set of common priming-dose genes (Tables 1 and 2). The over 10-fold up-regulation and over 10-fold

TABLE 5
Genes Associated with the Adaptive Outcome with Higher Expression in the
Adapting Cell Lines (Group 4)

Gene	Accession number	Relative expression			Ratio of effects ^a
		GM15036	GM15510	GM15268	
<i>NKTR</i>	NM_005385	0.2	6.9	4.3	28.0
<i>PSMC6</i>	D78275	0.2	1.9	1.2	7.8
<i>PFTK1</i>	AB020641	0.2	1.5	1.3	7.0
<i>MPHOSPH10</i>	X98494	0.5	3.2	2.5	5.7
<i>MTIF2</i>	AF494407	0.3	1.5	1.6	5.2
<i>CRM1</i>	Y08614	0.2	1.1	0.9	5.0
<i>RDX</i>	L02320	0.2	0.9	1.1	5.0
<i>TCF12</i>	M80627	0.3	1.2	1.8	5.0
<i>CETN3</i>	AI056696	0.5	2.1	2.6	4.7
<i>FLJ20720</i>	FLJ20719	1.1	5.9	4.1	4.5
<i>PSMA6</i>	X59417	0.3	1.7	0.9	4.3
<i>ZNF148</i>	AJ236885	0.3	1.8	0.8	4.3
<i>C2F</i>	U72514	0.5	2.8	1.5	4.3
<i>ERCC5</i>	L20046	0.5	2.8	1.5	4.3
<i>SMARCA2</i>	X72889	0.2	1	0.7	4.3
<i>DDX42</i>	AB036090	0.4	1.4	1.6	3.8
<i>HSPA8</i>	P11142	0.8	3.1	2.7	3.6
<i>EIF2S1</i>	BC002513	0.5	1.8	1.7	3.5
<i>EBNA1BP2</i>	U86602	0.6	2.3	1.8	3.4
<i>DKFZP564F0522</i>	AK027432	0.5	1.9	1.5	3.4

^a Calculated ratio of relative expression between non-adaptive and adaptive cell lines by averaging the results for the two adaptive cell lines.

ilar responses across all three cell lines irrespective of their adaptive response outcomes, indicate that protein biosynthesis alone is not a critical regulator of radioadaptation, as was previously suggested (26, 27).

The group 2 down-regulated metabolism genes code for proteins that are membrane-bound or related to mitochondrial processes (e.g. *ATP1B3*, *CYP1B1*, *SLC2A5* and *SLC9A6*; Table 3). In yeast, mitochondrial-related processes are known to be modulated after low-dose irradiation (44), and changes in the transcript levels of metabolic enzymes involved in oxidation/reduction reactions have been previously shown to be part of a low-dose mitochondrial stress response (50). Thus the general reduction in gene transcripts for metabolic enzymes may be related to an overall decrease in the oxidative capacity of cells that had received a priming dose compared to those that did not. Two genes associated with cell cycle control and DNA repair (*WEE1* and *PRKARIA*/protein kinase, cAMP-dependent) were down-regulated while *RBI* was up-regulated, suggesting complex roles for cell cycle control in cells that received the priming dose.

Previous to our study, the pathways that control adaptive response were inferred only from studies of individual transcripts and proteins. Using gene chips with cell lines that adapted or not, we identified several hundred candidate genes with differential transcript expression levels associated with adaptive response outcomes. These genes are diverse in their putative functions (Table 3), involving DNA repair, cell cycle control, chemokines and apoptosis as well as transcription and translation. Accordingly, our data suggest that the regulation of adaptive response may involve groups of genes (rather than individuals) whose regulation is juxtaposed, depending on whether the cells will adapt or not (Fig. 3, group 3 and 4). This observation is illustrated in Fig. 4 for DNA repair, stress responses, and proliferative and apoptosis pathways. Our findings predict that cells will adapt (i.e. show less chromosomal damage) when DNA repair and stress response genes are up-regulated at the same time that certain apoptosis and cell cycle control genes are down-regulated. Alternatively, cells will not adapt (i.e. show no change in the amount of chromosomal damage) when the gene transcript balance is shifted in the other direction (i.e., DNA repair and stress response genes are down-regulated, while apoptosis and cell cycle control genes are up-regulated).

Our microarray results point to a critical role for TP53-related pathways in the control of the adaptive response phenomenon and are consistent with several prior investigations of individual proteins in these pathways. TP53-related pathways have been implicated in adaptive response by affecting both DNA repair and apoptosis pathways related to functional TP53 (33, 51). Our microarray data suggest the involvement of DNA repair in adaptive response through the up-regulation of *ERCC5* and *ATM*. The *ERCC5* protein is involved in transcription-coupled repair of oxidative damage and nucleotide excision repair (52–54) and

has also been associated with adaptive response (55). *ATM* connects DSB recognition with modulation of TP53 functions (56). Prior studies with *ATM* null mice (34) suggested that *ATM* is not involved in adaptive response, which may be due to ATR complementing the *ATM* functions of null mice (57). Our microarray data suggest that *ATM* up-regulated transcripts are associated with adaptive response outcomes in lymphoblastoid cell lines, possibly for enhancing repair in response to radiation-induced DNA damage (58) through TP53 phosphorylation for cell cycle arrest or cell death by apoptosis (59, 60).

Our microarray data identify two stress-related processes related to adaptive response: chromatin remodeling and heat-shock responses (61, 62), both of which are related to TP53 function. *CBF2* was up-regulated in cells that adapted (Table 3), and the *CBF2* protein can interact with TP53 and P73 to modulate *HMG1* gene expression through changes in chromatin structure (63). The *HSP70* genes that are known to be involved in a radiation-inducible stress response mechanism (64, 65) were up-regulated in cells that adapted (Table 3). Induction of *HSP70* genes prior to stress exposures has been reported to suppress *TP53* expression, greatly decrease *BAX* levels, and inhibit apoptosis (66–68). In a prior study of *HSP70* responses under adaptive conditions, *HSPA8* transcript levels were not associated with adaptive responses in mouse splenocytes (69). However, this experiment failed to show induction of another *HSP70* family member, *PBP74*, which was previously associated with adaptive response (65), suggesting that there may be cell-type and tissue-specific variations in the genes associated with adaptive response. Our microarray data suggest that *HSP70* response mechanisms are critical components of the control of adaptive response in human lymphoblastoid cells.

Our data also implicate TP53-related cell cycle control and apoptosis functions in the control of adaptive response (Fig. 4). Example genes include *MYC*, *JUND*, *TNF*, *NFKB*, *CASP8*, *STAT1* and *STAT3*, which generally showed decreased levels in adapting cells compared to non-adapting cells. *MYC* is an important link in the control of cell cycle proliferation and apoptosis. It is a principal determinant in the TP53 DNA damage pathway (70, 71), regulating various interactions such as the transcriptional regulation of both *CDKN1A* (p21/CIP1) (72). Such interactions could prevent cell cycle arrest, which may be needed for efficient DNA repair processes. Others have also observed a down-regulation of the *MYC* transcript after irradiation (23), which is consistent with the suggestion that cells normally down-regulate *MYC* to enhance cell survival in response to genotoxic stress (73). Alternatively, induced levels of the *MYC* protein and transcript may lead to genomic instability and/or apoptosis (74, 75). Our observed transcript changes for *TNF* may also implicate cellular apoptosis in the adaptive response. The *TNF* protein is associated with activation of *NFKB*, *CASP8*, *STAT1* and *STAT3*, all of which can affect entry into the cell cycle and apoptosis. Down-regu-

lating TNF protein and other small cytokines may be important for maintaining cell cycle arrest (76–78), which is also likely to be important for efficient DNA repair.

Our study design has several notable strengths. The large number of genes assayed enabled the discovery of new genes, new groupings of genes, and complex patterns of transcription in response to radiation. The transcription analyses were performed on cells obtained from within the same experiments assayed for micronucleus frequencies to assess radioadaptive capacity of the cells; this nested design was critical because cell lines do not consistently show adaptation (13). Our approach to normalizing arrays and selecting subsets of potential genes for further evaluation is based on statistical methods developed to analyze and filter data from large expression arrays in a realistic and understandable way (79, 80). Such an approach allowed us to rank the genes in order of interest using techniques with known, predictable properties and behavior. The comparative tools EASE and GO provided insight into the underlying pathways and functions associated with the common priming dose effects and with the adaptive response outcomes.

Our study design has several limitations that will require further study. We used micronucleus frequency as the measure of adaptive response outcome, and it remains to be determined whether our findings will be applicable to other measures of adaptation, such as cell survival and genomic instability. A small number of cell lines were studied and only one time was evaluated. Further research is needed to determine how generalizable the results will be to whole organisms and other cell types. Specifically, epithelial cells tend to undergo growth arrest after radiation exposure, whereas lymphoid cells tend to undergo TP53-dependent apoptosis (81). Also, the use of GO categories was problematic because of the multiplicity of functions that can be assigned to any one protein, making it difficult to ascribe a single pathway or function to a gene. For example, *TNF* and *MYC* were identified within multiple maps such as Hs_cell, Hs_response to wounding 2, Hs_response to viruses, and Hs_response to biotic stimulus 5, as well as others (Table 3). Also, it remains essentially unknown how transcript changes (up or down) for genes are related to changes in protein levels, protein modifications, or cell fate. For some genes we already have evidence of protein changes associated with transcript changes. For example, transcript findings for *HSPA8* and *HSPD1* may be associated with changes in protein levels, since HSP70 transcription has been shown to correlate with increased HSP70 protein levels after radiation exposures (69, 82, 83).

In summary, our findings support our hypotheses. Exposures to 5-cGy priming doses led to changes in transcription that persisted beyond the much larger challenge dose. Two broad categories of primer-dose responsive genes were found: (1) genes with common responses after the priming dose, independent of whether the cell lines showed a cytogenetic adaptive response or not (the major effects were

up-regulation of genes associated with protein synthesis and down-regulation of metabolism genes) and (2) genes whose transcripts were differentially expressed in accordance with whether the cells subsequently adapt or not (the adaptive response appeared to be associated with differential expression of diverse genes, and we proposed that it is controlled in part by a balance between two sets of TP53-linked pathways: DNA repair/stress response genes and cellular proliferation/apoptosis genes). Our study findings also generate new hypotheses. Further work will be needed to determine whether low-dose-induced transcript alterations are associated with protein changes and whether controlling the expression of genes in the underlying pathways will correspondingly alter survival and residual genomic damage. Further studies will also be needed to determine whether the same pathways regulate low-dose-induced adaptive response in tumor cells *in vitro* and *in vivo* (e.g. 51, 84). This may lead to new insights and technologies for managing and controlling the consequences of exposure to ionizing radiation in radiotherapy, from occupational exposures, and after unexpected radiation exposure incidents.

ACKNOWLEDGMENTS

We would like to thank Irene Jones, Francesco Marchetti and Larry Thompson for their helpful suggestions in the preparation of this manuscript. We would also like to thank Aaron Biedma for help with bioinformatics. This work was performed under the auspices of the U.S. DOE by the University of California, LLNL contract W-7405-ENG-48 with funding from the U.S. DOE Low Dose Radiation Research Program, Biological and Environmental Research (BER), U.S. DOE grant KP110202.

Received: June 22, 2004; accepted: January 4, 2005

REFERENCES

1. E. Yin, D. O. Nelson, M. A. Coleman, L. E. Peterson and A. J. Wyrobek, Gene expression changes in mouse brain after exposure to low-dose ionizing radiation. *Int. J. Radiat. Biol.* **79**, 759–775 (2003).
2. W. Y. Park, C. I. Hwang, C. N. Im, M. J. Kang, J. H. Woo, J. H. Kim, Y. S. Kim, H. Kim, K. A. Kim and J. S. Seo, Identification of radiation-specific responses from gene expression profile. *Oncogene* **21**, 8521–8528 (2002).
3. S. A. Amundson, R. A. Lee, C. A. Koch-Paiz, M. L. Bittner, P. Meltzer, J. M. Trent and A. J. Fornace, Jr., Differential responses of stress genes to low dose-rate gamma irradiation. *Mol. Cancer Res.* **1**, 445–452 (2003).
4. S. Wolff, Aspects of the adaptive response to very low doses of radiation and other agents. *Mutat. Res.* **358**, 135–142 (1996).
5. G. Olivieri, J. Bodycote and S. Wolff, Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. *Science* **223**, 594–597 (1984).
6. H. Tuschl and R. Kovac, Sister chromatid exchanges (SCEs) in lymphocytes of persons working at Shlobin (USSR), 150 km north of Chernobyl. *Acta Biol. Hung.* **41**, 249–255 (1990).
7. N. Yoshida, H. Imada, N. Kunugita and T. Norimura, Low dose radiation-induced adaptive survival response in mouse spleen T-lymphocytes *in vivo*. *J. Radiat. Res.* **34**, 269–276 (1993).
8. S. Chen, L. Cai, X. Li and S. Liu, Low-dose whole-body irradiation induces alteration of protein expression in mouse splenocytes. *Toxicol. Lett.* **105**, 141–152 (1999).
9. I. Mosse, L. Kostrova, S. Subbot, I. Maksimenya and V. Molophei,

- Melanin decreases clastogenic effects of ionizing radiation in human and mouse somatic cells and modifies the radioadaptive response. *Radiat. Environ. Biophys.* **39**, 47–52 (2000).
10. W. Yu, M. Wang, L. Cai and Y. Jin, Pre-exposure of mice to low dose or low dose rate ionizing radiation reduces chromosome aberrations induced by subsequent exposure to high dose of radiation or mitomycin C. *Chin. Med. Sci. J.* **10**, 50–53 (1995).
 11. S. Venkat, S. K. Apte, R. C. Chaubey and P. S. Chauhan, Radioadaptive response in human lymphocytes *in vitro*. *J. Environ. Pathol. Toxicol. Oncol.* **20**, 165–175 (2001).
 12. G. D. Zasukhina, Radioadaptive response in human cells with different DNA repair activity. *Radiat. Biol. Radioecol.* **39**, 58–63 (1999).
 13. K. J. Sorensen, C. M. Attix, A. T. Christian, A. J. Wyrobek and J. D. Tucker, Adaptive response induction and variation in human lymphoblastoid cell lines. *Mutat. Res.* **519**, 15–24 (2002).
 14. M. S. Sasaki, On the reaction kinetics of the radioadaptive response in cultured mouse cells. *Int. J. Radiat. Biol.* **68**, 281–291 (1995).
 15. Z. Farooqi and P. C. Kesavan, Low-dose radiation-induced adaptive response in bone marrow cells of mice. *Mutat. Res.* **302**, 83–89 (1993).
 16. K. Ohtaki, H. Shimba, A. A. Awa and T. Sofuni, Comparison of type and frequency of chromosome aberrations by conventional and G-staining methods in Hiroshima atomic bomb survivors. *J. Radiat. Res.* **23**, 441–449 (1982).
 17. S. A. Amundson, M. Bittner, Y. Chen, J. Trent, P. Meltzer and A. J. Fornace, Jr., Fluorescent cDNA microarray hybridization reveals complexity and heterogeneity of cellular genotoxic stress. *Oncogene* **18**, 3666–3672 (1999).
 18. C. M. Kang, K. P. Park, J. E. Song, D. I. Jeoung, C. K. Cho, T. H. Kim, S. Bae, S. J. Lee and Y. S. Lee, Possible biomarkers for ionizing radiation exposure in human peripheral blood lymphocytes. *Radiat. Res.* **159**, 312–319 (2003).
 19. W. H. McBride, F. Pajonk, C. S. Chiang and J. R. Sun, NF- κ B, cytokines, proteasomes, and low-dose radiation exposure. *Mil. Med.* **167**, 66–67 (2002).
 20. B. E. Lehnert and R. Iyer, Exposure to low-level chemicals and ionizing radiation: Reactive oxygen species and cellular pathways. *Hum. Exp. Toxicol.* **21**, 65–69 (2002).
 21. N. F. Marko, P. B. Dieffenbach, G. Yan, S. Ceryak, R. W. Howell, T. A. McCaffrey and V. W. Hu, Does metabolic radiolabeling stimulate the stress response? Gene expression profiling reveals differential cellular responses to internal beta vs. external gamma radiation. *FASEB J.* **17**, 1470–1486 (2003).
 22. S. A. Amundson, K. T. Do and A. J. Fornace, Jr., Induction of stress genes by low doses of gamma rays. *Radiat. Res.* **152**, 225–231 (1999).
 23. V. G. Tusher, R. Tibshirani and G. Chu, Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl. Acad. Sci. USA* **98**, 6635–6640 (2001).
 24. M. A. Chaudhry, L. A. Chodosh, W. G. McKenna and R. J. Muschel, Gene expression profile of human cells irradiated in G₁ and G₂ phases of cell cycle. *Cancer Lett.* **195**, 221–233 (2003).
 25. A. Sreekumar, M. K. Nyati, S. Varambally, T. R. Barrette, D. Ghosh, T. S. Lawrence and A. M. Chinnaiyan, Profiling of cancer cells using protein microarrays: discovery of novel radiation-regulated proteins. *Cancer Res.* **61**, 7585–7593 (2001).
 26. J. H. Youngblom, J. K. Wiencke and S. Wolff, Inhibition of the adaptive response of human lymphocytes to very low doses of ionizing radiation by the protein synthesis inhibitor cycloheximide. *Mutat. Res.* **227**, 257–261 (1989).
 27. J. H. Kim, K. H. Hahm, C. K. Cho and S. Y. Yoo, Protein biosynthesis in low dose ionizing radiation-adapted human melanoma cells. *J. Radiat. Res.* **37**, 161–169 (1996).
 28. K. P. Guruprasad and V. Vasudev, Inducible protective processes in animal systems: VIII. Enhancement of adaptive response by nicotine. *Mutagenesis* **16**, 257–263 (2001).
 29. B. Marples, B. G. Wouters, S. J. Collis, A. J. Chalmers and M. C. Joiner, Low-dose hyper-radiosensitivity: A consequence of ineffective cell cycle arrest of radiation-damaged G₂-phase cells. *Radiat. Res.* **161**, 247–255 (2004).
 30. J. D. Shadley, V. Afzal and S. Wolff, Characterization of the adaptive response to ionizing radiation induced by low doses of X rays to human lymphocytes. *Radiat. Res.* **111**, 511–517 (1987).
 31. I. Szumiel, Monitoring and signaling of radiation-induced damage in mammalian cells. *Radiat. Res.* **150** (Suppl.), S92–S101 (1998).
 32. R. Sasaki, T. Shirakawa, Z. J. Zhang, A. Tamekane, A. Matsumoto, K. Sugimura, M. Matsuo, S. Kamidono and A. Gotoh, Additional gene therapy with Ad5CMV-p53 enhanced the efficacy of radiotherapy in human prostate cancer cells. *Int. J. Radiat. Oncol. Biol. Phys.* **51**, 1336–1345 (2001).
 33. M. S. Sasaki, Y. Ejima, A. Tachibana, T. Yamada, K. Ishizaki, T. Shimizu and T. Nomura, DNA damage response pathway in radioadaptive response. *Mutat. Res.* **504**, 101–118 (2002).
 34. T. Robson, M. C. Joiner, G. D. Wilson, W. McCullough, M. E. Price, I. Logan, H. Jones, S. R. McKeown and D. G. Hirst, A novel human stress response-related gene with a potential role in induced radioresistance. *Radiat. Res.* **152**, 451–461 (1999).
 35. T. Robson, M. E. Price, M. L. Moore, M. C. Joiner, V. J. McKelvey-Martin, S. R. McKeown and D. G. Hirst, Increased repair and cell survival in cells treated with DIR1 antisense oligonucleotides: Implications for induced radioresistance. *Int. J. Radiat. Biol.* **76**, 617–623 (2000).
 36. S. Grosch, G. Fritz and B. Kaina, Apurinic endonuclease (Ref-1) is induced in mammalian cells by oxidative stress and involved in clastogenic adaptation. *Cancer Res.* **58**, 4410–4416 (1998).
 37. R. Ihaka and R. Gentleman, A language for data analysis and graphics. *J. Comput. Graph. Stat.* **5**, 299–314 (1996).
 38. S. Dudoit, J. Popper-Shaffer and J. C. Boldrick, *Multiple Hypothesis Testing in Microarray Experiments*. UC Berkeley Division of Biostatistics Working Paper Series, 2002. [Available online at <http://www.bepress.com/ucbbiostat/paper110>]
 39. S. W. Doniger, N. Salomonis, K. D. Dahlquist, K. Vranizan, S. C. Lawlor and B. R. Conklin, MAPFinder: Using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. *Genome Biol.* **4**, R7 (2003).
 40. M. Ashburner, C. A. Ball, J. A. Blake, D. Botstein, H. Butler, J. M. Cherry, A. P. Davis, K. Dolinski, S. S. Dwight and G. Sherlock, Gene ontology: Tool for the unification of biology. The Gene Ontology Consortium. *Nat. Genet.* **25**, 25–29 (2000).
 41. L. M. Tomascik-Cheeseman, M. A. Coleman, F. Marchetti, D. O. Nelson, L. M. Kegelmeyer, J. Nath and A. J. Wyrobek, Differential basal expression of genes associated with stress response, damage control, and DNA repair among mouse tissues. *Mutat. Res.* **561**, 1–14 (2004).
 42. L. E. Peterson, CLUSFAVOR 5.0: hierarchical cluster and principal-component analysis of microarray-based transcriptional profiles. *Genome Biol.* **3**, SOFTWARE0002 (2002).
 43. M. B. Eisen, P. T. Spellman, P. O. Brown and D. Botstein, Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. USA* **95**, 14863–14868 (1998).
 44. G. Mercier, N. Berthault, J. Mary, J. Peyre, A. Antoniadis, J. P. Comet, A. Cornuejols, C. Froidevaux and M. Dutreix, Biological detection of low radiation doses by combining results of two microarray analysis methods. *Nucleic Acids Res.* **32**, e12 (2004).
 45. L. Xia, A. Paik and J. J. Li, p53 activation in chronic radiation-treated breast cancer cells: regulation of MDM2/p14ARF. *Cancer Res.* **64**, 221–228 (2004).
 46. Z. Li, L. Xia, L. M. Lee, A. Khaletskiy, J. Wang, J. Y. Wong and J. J. Li, Effector genes altered in MCF-7 human breast cancer cells after exposure to fractionated ionizing radiation. *Radiat. Res.* **155**, 543–553 (2001).
 47. S. A. Amundson, A. Patterson, K. T. Do and A. J. Fornace, Jr., A nucleotide excision repair master-switch: p53 regulated coordinate induction of global genomic repair genes. *Cancer Biol. Ther.* **1**, 145–149 (2002).

48. M. S. Sheikh and A. J. Fornace, Regulation of translation initiation following stress. *Oncogene* **18**, 6121–6128 (1999).
49. K. Y. Jen and V. G. Cheung, Transcriptional response of lymphoblastoid cells to ionizing radiation. *Genome Res.* **13**, 2092–2100 (2003).
50. D. W. Voehringer, D. L. Hirschberg, J. Xiao, Q. Lu, M. Roederer, C. B. Lock, L. A. Herzenberg and L. Steinman, Gene microarray identification of redox and mitochondrial elements that control resistance or sensitivity to apoptosis. *Proc. Natl. Acad. Sci. USA* **97**, 2680–2685 (2000).
51. R. E. J. Mitchel, J. S. Jackson and S. M. Carlisle, Upper dose thresholds for radiation-induced adaptive response against cancer in high-dose-exposed, cancer-prone, radiation-sensitive Trp53 heterozygous mice. *Radiat. Res.* **162**, 20–30 (2004).
52. P. K. Cooper, T. Nospikel, S. G. Clarkson and S. A. Leadon, Defective transcription-coupled repair of oxidative base damage in Cockayne syndrome patients from XP group G. *Science* **275**, 990–993 (1997).
53. R. D. Wood, S. J. Araujo, R. R. Ariza, D. P. Batty, M. Biggerstaff, E. Evans, P. H. Gaillard, D. Gunz, B. Koberle and M. K. Shivji, DNA damage recognition and nucleotide excision repair in mammalian cells. *Cold Spring Harb. Symp. Quant. Biol.* **65**, 173–182 (2000).
54. P. C. Hanawalt, Subpathways of nucleotide excision repair and their regulation. *Oncogene* **21**, 8949–8956 (2002).
55. M. Weinfeld, J. Z. Xing, J. Lee, S. A. Leadon, P. K. Cooper and X. C. Le, Factors influencing the removal of thymine glycol from DNA in gamma-irradiated human cells. *Prog. Nucleic Acid Res. Mol. Biol.* **68**, 139–149 (2001).
56. M. B. Kastan, ATM—the first step in helping cells deal with DNA damage. *Biomed. Pharmacother.* **58**, 72–73 (2004).
57. N. J. Bentley, D. A. Holtzman, G. Flaggs, K. S. Keegan, A. DeMaggio, J. C. Ford, M. Hoekstra and A. M. Carr, The *Schizosaccharomyces pombe* rad3 checkpoint gene. *EMBO J.* **15**, 6641–6651 (1996).
58. C. J. Bakkenist and M. B. Kastan, DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* **421**, 499–506 (2003).
59. N. D. Lakin and S. P. Jackson, Regulation of p53 in response to DNA damage. *Oncogene* **18**, 7644–7655 (1999).
60. A. N. Heinloth, R. E. Shackelford, C. L. Innes, L. Bennett, L. Li, R. P. Amin, S. O. Sieber, K. G. Flores, P. R. Bushel and R. S. Paules, ATM-dependent and -independent gene expression changes in response to oxidative stress, gamma irradiation, and UV irradiation. *Radiat. Res.* **160**, 273–290 (2003).
61. K. Matuoka and K. Y. Chen, Transcriptional regulation of cellular ageing by the CCAAT box-binding factor CBF/NF-Y. *Ageing Res. Rev.* **1**, 639–651 (2002).
62. S. N. Agoff, J. Hou, D. I. Linzer and B. Wu, Regulation of the human hsp70 promoter by p53. *Science* **259**, 84–87 (1993).
63. H. Uramoto, H. Izumi, G. Nagatani, H. Ohmori, N. Nagasue, T. Ise, T. Yoshida, K. Yasumoto and K. Kohno, Physical interaction of tumour suppressor p53/p73 with CCAAT-binding transcription factor 2 (CTF2) and differential regulation of human high-mobility group 1 (HMG1) gene expression. *Biochem. J.* **371**, 301–310 (2003).
64. S. J. Lee, S. A. Choi, K. H. Lee, H. Y. Chung, T. H. Kim, C. K. Cho and Y. S. Lee, Role of inducible heat shock protein 70 in radiation-induced cell death. *Cell Stress Chaperones* **6**, 273–281 (2001).
65. S. Sadekova, S. Lehnert and T. Y. Chow, Induction of PBP74/mortalin/Grp75, a member of the hsp70 family, by low doses of ionizing radiation: a possible role in induced radioresistance. *Int. J. Radiat. Biol.* **72**, 653–660 (1997).
66. S. A. Gordon, R. A. Hoffman, R. L. Simmons and H. R. Ford, Induction of heat shock protein 70 protects thymocytes against radiation-induced apoptosis. *Arch Surg.* **32**, 1277–1282 (1997).
67. B. Bailly-Maitre, G. de Sousa, N. Zucchini, J. Gugenheim, K. E. Boulukos and R. Rahmani, Spontaneous apoptosis in primary cultures of human and rat hepatocytes: Molecular mechanisms and regulation by dexamethasone. *Cell Death Differ.* **9**, 945–955 (2002).
68. R. Terauchi, K. A. Takahashi, Y. Arai, T. Ikeda, S. Ohashi, J. Imanishi, O. Mazda and T. Kubo, Hsp70 prevents nitric oxide-induced apoptosis in articular chondrocytes. *Arthritis Rheum.* **48**, 1562–1568 (2003).
69. C. M. Kang, K. P. Park, C. K. Cho, J. S. Seo, W. Y. Park, S. J. Lee and Y. S. Lee, Hspa4 (HSP70) is involved in the radioadaptive response: Results from mouse splenocytes. *Radiat. Res.* **157**, 650–655 (2002).
70. K. H. Maclean, U. B. Keller, C. Rodriguez-Galindo, J. A. Nilsson and J. L. Cleveland, c-Myc augments gamma irradiation-induced apoptosis by suppressing Bcl-XL. *Mol. Cell Biol.* **23**, 7256–7270 (2003).
71. J. Seoane, H. V. Le and J. Massague, Myc suppression of the p21(Cip1) Cdk inhibitor influences the outcome of the p53 response to DNA damage. *Nature* **419**, 729–734 (2002).
72. S. Wu, C. Cetinkaya, M. J. Munoz-Alonso, N. von der Lehr, F. Bahram, V. Beuger, M. Eilers, J. Leon and L. G. Larsson, Myc represses differentiation-induced p21CIP1 expression via Miz-1-dependent interaction with the p21 core promoter. *Oncogene* **22**, 351–360 (2003).
73. K. J. Magnet, M. S. Orr, J. L. Cleveland, C. Rodriguez-Galindo, H. Yang, C. Yang, Y. M. Di, P. T. Jain and D. A. Gewirtz, Suppression of c-myc expression and c-Myc function in response to sustained DNA damage in MCF-7 breast tumor cells. *Biochem. Pharmacol.* **62**, 593–602 (2001).
74. J. H. Sheen, J. K. Woo and R. B. Dickson, c-Myc alters the DNA damage-induced G₂/M arrest in human mammary epithelial cells. *Br. J. Cancer* **89**, 1479–1485 (2003).
75. J. H. Sheen and R. B. Dickson, Overexpression of c-Myc alters G₂/S arrest following ionizing radiation. *Mol. Cell Biol.* **22**, 1819–1833 (2002).
76. D. Sarkar, M. Leszczyniecka, D. C. Kang, I. V. Lebedeva, K. Valerie, S. Dhar, T. K. Pandita and P. B. Fisher, Down-regulation of Myc as a potential target for growth arrest induced by human polynucleotide phosphorylase (hPNPaseold-35) in human melanoma cells. *J. Biol. Chem.* **278**, 24542–24551 (2003).
77. B. J. Warner, S. W. Blain, J. Seoane and J. Massague, Myc down-regulation by transforming growth factor beta required for activation of the p15(Ink4b) G₁ arrest pathway. *Mol. Cell Biol.* **19**, 5913–5922 (1999).
78. K. Wakasugi, B. M. Slike, J. Hood, A. Otani, K. L. Ewalt, M. Friedlander, D. A. Cheresh and P. Schimmel, A human aminoacyl-tRNA synthetase as a regulator of angiogenesis. *Proc. Natl. Acad. Sci. USA* **99**, 173–177 (2002).
79. B. M. Bolstad, R. A. Irizarry, M. Astrand and T. P. Speed, A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* **19**, 185–193 (2003).
80. R. A. Irizarry, B. Hobbs, F. Collin, Y. D. Beazer-Barclay, K. J. Antonellis, U. Scherf and T. P. Speed, Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4**, 249–264 (2003).
81. A. R. Pettitt, P. D. Sherrington, G. Stewart, J. C. Cawley, A. M. Taylor and T. Stankovic, p53 dysfunction in B-cell chronic lymphocytic leukemia: Inactivation of ATM as an alternative to TP53 mutation. *Blood* **98**, 814–822 (2001).
82. S. H. Park, S. J. Lee, H. Y. Chung, T. H. Kim, C. K. Cho, S. Y. Yoo and Y. S. Lee, Inducible heat-shock protein 70 is involved in the radioadaptive response. *Radiat. Res.* **153**, 318–326 (2000).
83. K. Suzuki, S. Kodama and M. Watanabe, Effect of low-dose pre-irradiation on induction of the HSP70B-LacZ fusion gene in human cells treated with heat shock. *Radiat. Res.* **149**, 195–201 (1998).
84. E. I. Azzam, G. P. Raaphorst and R. E. J. Mitchel, Radiation-induced adaptive response for protection against micronucleus formation and neoplastic transformation in C3H 10T^{1/2} mouse embryo cells. *Radiat. Res.* **138** (Suppl.), S28–S31 (1994).