**Low Dose Radiation Response Curves, Networks and Pathways in Human Lymphoblastoid
Cells Exposed from 1 to 10 cGy of Acute Gamma Radiation**

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Running head: Low-dose transcript profiles in human blood-derived cells

Abstract

We investigated the low dose dependency of the transcriptional response of human cells to characterize the shape and biological functions associated with the dose response curve and to identify common and conserved functions of low dose expressed genes across cells and tissues. Human lymphoblastoid (HL) cells from two unrelated individuals were exposed to graded doses of radiation spanning the range of 1-10 cGy were analyzed by transcriptome profiling, qPCR and bioinformatics, in comparison to sham irradiated samples. A set of ~80 genes showed consistent responses in both cell lines; these genes were associated with homeostasis mechanisms (e.g., membrane signaling, molecule transport), subcellular locations (e.g., Golgi, and endoplasmic reticulum), and involved diverse signal transduction pathways. The majority of radiation-modulated genes had plateau-like responses across 1-10 cGy, some with suggestive evidence that transcription was modulated at doses below 1 cGy. MYC, FOS and TP53 were the major network nodes of the low-dose response in HL cells. Comparison our low dose expression findings in HL cells with those of prior studies in mouse brain after whole body exposure, in human keratinocyte cultures, and in endothelial cells cultures, indicates that certain components of the low dose radiation response are broadly conserved across cell types and tissues, independent of proliferation status.

Key Words: Gene expression, transcripts, low dose ionizing radiation, human lymphoblastoid cells, gene networks

Microarray data are submitted to the Gene Expression Omnibus Database
(<http://www.ncbi.nlm.nih.gov/geo/>).

Introduction

Human exposures to low-dose ionizing radiation (from natural sources, cosmic rays, nuclear power and various sources of radioactive waste) are far more common than exposure to high doses. Tissue damage and cancer risks after exposures to high-dose ionizing radiation are well documented, but there is considerable uncertainty about health risks that may be associated with exposures to low doses, i.e., < 10 cGy [1,2]. Exposure of normal tissue to low-dose ionizing radiation is becoming increasingly commonplace in medicine and dentistry [3]. Epidemiological evidence suggests that exposures of 0.2 - 3.0 Sv increase the risks for cancer and other ill health effects [4]. However, the limitations of epidemiological extrapolations into the lower dose range have sparked vigorous scientific and public policy controversies [2,5]. The latest “Biological Effects of Ionizing Radiation” (BEIR VII) report concluded that the available biological and biophysical data remain consistent with a “linear, no-threshold” (LNT) risk model, especially for solid tumors. This model predicts that even the smallest doses of ionizing radiation (IR) will increase the risk for tumors [6]. In contrast, the French Academy of Sciences [2,7] questioned the acceptance of the Linear no-threshold (LNT) model for low-doses in light of the mounting evidence for non-linearity in biochemical and cellular effects after low-dose exposures [2,3,8-10]. Addressing this controversy is important because inappropriate estimates of low dose risks may lead to undesirable consequences, such as discouraging patients from undergoing useful medical examinations, limiting space flight, or requiring costly radioprotective measures.

High-dose exposures (>2 Gy) are known to modulate the expression of genes associated with genotoxic and physiological stress responses including DNA damage sensing and repair, and immune response [11-21]. After high doses, the cellular decisions to initiate rescue or cell death

appear to be mediated by signaling pathways and secondary messengers [22-24]. Several cytoplasmic pathways are rapidly activated, including cytoplasmic Ca^{2+} homeostasis mechanisms [25], reactive oxygen species induced responses [26], kinase cascades [27] and ceramide production [28]. There are associated increases in mitochondrial permeability and calcium release as well as reactive oxygen species [25,29,30]. DNA, cellular membranes, and organelles are important targets of high-dose radiation [26,31,32], but their roles after exposure to low doses are poorly understood.

Transcriptional profiling is a sensitive biomarker of radiation exposure and has been used to gain insight into the molecular mechanisms induced by low dose exposures in a variety of cell types: cell cultures of human myeloid cells [14,33], keratinocytes [34], lymphocytes [35], umbilical vein endothelial cells [36], lymphoblastoid cells [37], and mesenchymal stem cells [38]. Only a few studies investigated transcript profiles after *in vivo* low-dose exposures. Our study of brain tissue from irradiated mice [19] identified several hundred genes with modulated transcript levels after exposures of 10 cGy. Using skin biopsies from radiotherapy patients, Goldberg and colleagues identified low-dose genes, their variability and functional groups [39-41]. A proteomic survey identified several proteins that were phosphorylated or modulated at doses below 10 cGy in a variety of model systems [42]. These studies demonstrate that doses of 1 cGy of low-linear energy transfer (LET) radiation (~ 0.3 double strand breaks per cell) are sufficient to modulate gene expression. However, there is insufficient information on the shape and slope of the low dose response curves for gene expression and the associated molecular functions, both of which are needed to assess relevance of the LNT model [43].

The purpose of our study was to identify genes whose transcript expression was consistently modulated across the low-dose radiation range in lymphoblastoid cell lines from two unrelated individuals, to characterize the shape of the low dose response curve, identify the functions and gene-interaction networks of low dose radiation-sensitive genes, and identify the components of the low dose molecular responses that are conserved across selected human and mouse tissues.

Materials and Methods

Cell culture, irradiation, RNA extraction and microarray analyses

We investigated the transcription profiles in two independent human lymphoblastoid cell lines irradiated at 5 dose levels spanning the low-dose range of 1-10 cGy using Affymetrix microarrays (HG –U133A), containing 22,283 probes and a linear amplification procedure to improve the detection of low-abundance transcripts [44]. Two human lymphoblastoid cell lines (GM15036 and GM15510) from the Coriell Cell Repositories, were grown in suspension in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 15% heat inactivated fetal bovine serum (Sigma Aldrich), and 1X antibiotic-antimycotic (consisting of 100 units/ml penicillin G sodium, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B as Fungizone® in 0.85% saline; and 2 mM L-glutamine, Invitrogen). The Coriell Cell Repositories were selected from the genetic diversity set as representative of normal individuals within the U.S.A. These cell lines are used because they are EBV immortalized cells and contain normal diploid karyotypes. Cell cultures were grown in a humidified 5% CO₂ atmosphere at 37 °C, maintained at $1-10 \times 10^5$ cells/mL. Ten mL of culture at $2-3 \times 10^5$ cells/mL in T-25 flasks received fresh media 24 hours before irradiation. Approximately 5×10^6 cells were irradiated in 37 °C media using a ¹³⁷Cs Mark 1 Irradiator (J.L. Shepherd and Associates, Glendale, CA) to deliver 0 (sham) 1, 2.5, 5, 7.5 and 10 cGy (dose rates: 1.5-4 cGy/min). Following irradiation, cells were incubated at 37 °C and harvested 4 hours later. Cultures were centrifuged, pellets washed with phosphate buffered saline, resuspended in 1 mL of cold culture medium, transferred to a 2 mL cryotube, flash frozen in liquid nitrogen, and stored at -80 °C. Total RNA was extracted from thawed cells (RNeasy Total RNA Isolation Kit according to the manufacturer's protocol; QIAGEN, Valencia, CA) and treated with RNase-free DNase during the isolation procedure to remove contaminating genomic

DNA (QIAGEN, Valencia, CA). RNA integrity and quantity were confirmed by agarose gel electrophoresis with ethidium bromide staining and spectrophotometry, or by using an Agilent 2100 Bioanalyzer and the RNA 6000 Nano Assay LabChip. Purified total RNA was stored at -80 °C until further use. This study consisted of 12 biological samples (2 cell lines x 6 doses including sham control) and 36 microarrays.

Total RNA from each sample was amplified using the ARCTURUS RiboAmp[®] kit (ARCTURUS Biosciences, Mountain View, CA). RNA amplification provides enhanced detection of low abundance transcripts while achieving greater than 70% agreement between transcripts detected with 1 or 2 rounds of amplification compared to no amplification at all. The processed RNA was then converted to double stranded cDNA, and the Enzo BioArray HighYield Transcript Kit was used for a single round of RNA amplification-based labeling (Enzo Biochem, New York, NY). After labeling, the antisense RNA was fragmented as described in the Affymetrix Gene Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA) and evaluated for quality and quantity on the Agilent 2100 Bioanalyzer prior to microarray hybridization. Thirty six Affymetrix Human Genome HG-U133A gene chips (2 cell lines to achieve biological replication, 6 doses, in triplicate) were hybridized using 15 µg of fragmented complementary RNA followed by washing and staining in an Affymetrix Fluidics Workstation as described in the Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA). Hybridized chips were scanned and signals detected using an argon-ion laser scanner (Agilent Technologies, Palo Alto, CA). Microarray data were assessed for hybridization quality, and individual CEL files were used for data preprocessing as described below. All microarray-related CEL files and corresponding information generated as part of these studies were deposited with

the Gene Expression Omnibus repository (<http://www.ncbi.nlm.nih.gov/geo/>) under accession number GSE22639.

Statistical Analyses

Selection of “candidate” and “consistent” sets of low-dose responsive genes: Fold changes for each gene were calculated across doses by fitting a simple linear model to the data, using 30 degrees of freedom (36 chips minus 6 doses) to estimate the noise. The p values were used to identify genes with significant hybridization signals, and were adjusted by the Benjamini-Hochberg method to control the per-chip false discovery rate (FDR), using the “mt.rawp2adjp” procedure in Bioconductor [45]. Only genes with an FDR-adjusted p value not exceeding 0.01 were selected. For each gene and cell line, t-statistics were computed for each log fold change. QQ-plots were used to compare the distribution of the t-statistics. Robust linear regression was used on the observed versus theoretical quantiles to determine what linear transformation of these t-statistics would confer a normal distribution, and then scaled accordingly. 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. First, the initial data set consisted of expression data for all the genes for which a signal was detected in at least one dose for that cell line. Second, the data from the two cell lines were combined into a single data set. The resulting data set consisted of genes that had significant radiation-modulated transcript expression in one or both cell lines for at least one of the doses tested. Differential expression across the six IR doses was detected by an F test, and a separate F test was performed on each gene and each cell line. After adjusting the p values for these genes using the Benjamini-Hochberg procedure, 291 genes (referred to as the “candidate set” of low-dose responsive genes, Table S1) showed an adjusted F

test p value of ≤ 0.05 indicating differential expression across the low dose range. A subset of 81 genes showed consistent significant responses in both cell lines (referred to as the “*consistent set*” of low-dose responsive genes; Table 1). We found 274 genes with annotations within the 291-gene set. Similarly, there were 75 annotated genes in the 81-gene set.

Dose response analyses: The expression profiles of the 81 gene consistent set were evaluated by linear regression analyses for each cell line independently, using the Linear Models for Microarray Data (LIMMA) package in the R statistical environment [46]. LIMMA was used to determine the fit of the linear model for fold change for each gene for each cell line, to obtain the 95% CI on the sham irradiated samples, and to determine the slope and intercept values with 95% CI of the regression line across the 5 dose values.

Pathways and network analysis

Gene annotations and functional classifications were assessed with EASE (www.david.gov/EASE; [47]) and the "Gene Ontology" (GO) database (www.geneontology.org; [48]), with screening for statistically overrepresented categories using Gostat (gostat.wehi.edu.au; [49]). Functional classifications and pathways were selected as statistically enriched above the background using the Affymetrix U133A chip. Pathway analyses were performed using Ingenuity Systems Pathway Knowledge Database (www.ingenuity.com). Genes from the two input lists (i.e., candidate and consistent gene lists) were mapped to distinct biological networks. Analyses were limited to networks with rank scores of >3 (i.e., $> 99.9\%$ chance of not being generated by random chance). Fischer exact p -values were used to determine the probability that a biological function assigned to a network occurred by chance. In addition,

we used the website for Information Hyperlinked Over Proteins (IHOP) to obtain description of protein interactions and linkages (www.ihop-net.org/UniPub/iHOP/).

We compared the networks and nodal genes with those identified in low dose transcript databases of three very diverse biological systems: human umbilical vein endothelial cells (HUVEC) [36], primary human keratinocytes (both irradiated in near confluency cultures) [34] and brain tissue from mice irradiated whole body [19,50]. The gene transcript data from HUVEC exposed to X-Rays in the dose range of 2-200 cGy were downloaded, and from this 308 modulated genes were selected which had >2 fold change up or down at 2 cGy at 4 hr time point. These were imported into Ingenuity to generate gene-gene interaction networks. Gene transcript data were downloaded for keratinocytes isolated from adult normal skin exposed to an X-ray dose of 1 cGy and sampled after 48 hrs; this data set yielded 121 modulated genes with a ≥ 1.5 fold change. We also used data from our own lab for transcripts from irradiated mouse brains [19,50]. These databases were imported into the Ingenuity pathway analysis tool for generating gene interaction networks. Nodal genes were defined as having direct connections with ≥ 5 genes that were modulated in their respective data sets. Ingenuity network scores were used to compare the significance of the networks that contained these nodes.

RT-PCR validation

Genes were selected based on an intensity filter (>500 signal intensity values and >1.5 average fold changes across doses) for evaluation by quantitative real-time reverse transcriptase PCR (QRT-PCR). First, 1-2 micrograms of total RNA and random primers were used to generate cDNA (High Capacity Archive Kit, Applied Biosystems, Inc. Foster City CA). Second, aliquots

of cDNA were used to perform PCR using TaqMan® Gene Expression Assays, with gene specific primers (900 nM each) and fluorescent probes. The TaqMan probes were specifically designed to span exon-exon junction regions of the respective genes of interest. The reactions were run on an ABI Sequence Detection System 7900HT Real-Time PCR System using a 384 well format, with both no-template and no-primer controls. The reaction conditions were as follows: (step 1) 95 °C for 1 minute, (step 2) 95 °C for 15 seconds, (step 3) 60 °C for 30 seconds, (step 4) cycle through steps 2 and 3 for an additional 39 times, and a final hold at 4°C. Efficiency of amplification for all primer probe sets was estimated to be >96% (Data not shown). Multiple endogenous controls including 18S, microglobulin, actin and GAPDH were analyzed (data not shown); GAPDH was determined to be the least variant and hence used as the endogenous control for relative quantification to generate the delta Ct values and fold changes in gene expression as previously described [51,52]. The normalized results for each of the four replicates of each tested gene were averaged and compared to unity and microarray fold changes, using confidence intervals (95% CI). The following gene-specific assays were used for Figure 1: CD164 (Hs00174789_m1), GGH (Hs00608257_m1), LIPA (Hs00426932_m1), PPT1 (Hs00165679_m1), SCAMP1 (Hs00792736_m1), SSR1 (Hs00162340_m1), PAM (Hs00168596_m1), ACSL3 (Hs00244853_m1). QRT-PCR data for 17 selected genes are shown in Table S2.

Results:

Microarray identification of low-dose responsive genes and RT-PCR confirmation

Analyses of the gene-transcript data yielded a “*candidate*” set of 291 genes with statistically significant transcript modulation in at least one of the two independent cell lines (GM15036 and GM15510) for at least one of the 5 doses tested: 1, 2.5, 5.0, 7.5, 10 cGy (Table S1 lists these 291 genes, FDR<0.05). Applying more stringent criteria [(a) average >1.5 fold increases across all doses tested within each cell line, and (b) the 95% confidence interval excluded a fold change value of unity] produced a set of 81 genes with consistent responses in both cell lines (i.e., “*consistent*” set), of which 80 genes were up-regulated and one was down-regulated (Table 1). Over 40% of these genes showed >1.8 fold response in both cell lines, and examples of “consistent” genes were validated using quantitative RT-PCR (Figure 1, Table S2).

Dose response curve and Y-intercept analyses of low-dose transcriptional responses

The dose dependency of transcript expression (slope and Y-intercept) in the 1-10 cGy range of exposures was investigated for the 81-gene set showing differential expression at 4 hours using linear regression analyses. Thioredoxin domain containing protein 13, (DJ971N18.2) showed significant positive slopes in both cell lines: 1.8 (95% CI, 1.3:2.3) and 1.8 (1.4:2.1) for lines GM15510 and GM15036, respectively ($p < 2E^{-10}$). Of the genes showing a statistically significant slope in only one cell line, 20 increased with dose and H2AFX decreased. The majority of “consistent” genes [53] did not have a statistically significant slope (up or down) in either cell line (i.e., findings that are consistent with flat, “plateau-like” responses in the 1 – 10 cGy range, and provides justification for using average changes across doses as a criterion for selecting the consistent and candidate gene sets, above). The regression lines of 16 genes had

significantly elevated Y intercepts in both cell lines, ranging from 2.6 to 3.5 fold changes for cell line GM15036, and 1.7 to 2.6 fold-changes for cell line GM15510, (Table 2, Y-intercepts, $p < 1.7E-11$). Consistent with these findings, several of these genes had statistically significant elevated fold changes at 1 cGy by pair-wise analysis against the sham controls ($p < 0.05$). Figure 2 illustrates the dose dependency with regression lines for two example genes for the two independent cell lines. This suggests that transcription of these genes may be induced at doses lower than 1 cGy. The microarray data also showed elevated transcript levels of several DNA damage response genes, such as CDKN1A, RAD54B, and GADD45A. Real time PCR confirmed these genes as dose responsive and not limited to the low dose range.

Cellular functions and locations of low-dose gene products

The genes of the low-dose gene sets were assigned to biological processes and cellular locations using gene ontology (GO) (Table 3). For the 291-gene candidate set, significant assignments ($p < 0.005$) were obtained for homeostasis functions (i.e., cellular homeostasis and ion homeostasis), localization functions (i.e., endomembrane system, endoplasmic reticulum) and transport functions (i.e., protein transport, amino acid transport, and cation transport). A second analysis limited to the 81-gene consistent set confirmed these findings (Table 3). The 291-gene candidate set was also highly associated ($p < 10E^{-11}$) with cellular locations including several membrane locations (i.e., integral to membrane, endoplasmic reticulum, nuclear envelope, organelle, membrane-bound vesicles, Golgi) and cytoplasmic locations, which again was confirmed by a second analysis with the 81-gene consistent set (Table 3). Tables S3-5 provide further details of the assignment of the low-dose genes to specific subcellular locations,

homeostasis functions, and signal transduction pathways based on Ingenuity, Gene Ontology, and EASE findings. Overall, ~30% of genes were assigned to cytoplasmic locations.

The genes assigned to homeostasis functions (Table S4) are associated with solute transport (cations and anions, 29 genes; amino acid and peptide, 13 genes), energy (11 genes), and metabolism (fatty acid, 19 genes; glycoprotein, 12 genes; carbohydrate, 2 genes; protein, 28 genes; RNA/DNA, 17 genes). More specifically, the solute-transport genes are involved with transport of metal ions, sodium and potassium, amino acids, nucleotides, glucose and fatty acids; e.g., SLC1A1 (glutamate), SLC39A14 (zinc ion), SLC25A3 (phosphate), SLC39A8 (zinc), RCN1 and RCN2 (calcium ion), SLC12A2 (sodium chloride / potassium chloride), SLC30A1 (hydrogen peroxide), SLC9A6 (sodium ion /proton).

Energy-associated genes were primarily involved in ATPase functions for small molecule membrane mediated transport, such as ATP1B3, ATP2C1, ATP11B, and ATP10D. Genes associated with cellular metabolism included ARL6IP5 (amino acid); ACSL1 and ACSL3 (fatty acid); MAN1A1 (carbohydrate metabolism), LIPA (lipid metabolism); GALNT7, TXNDC4, RPN1, RPN2 (glycoprotein metabolism), and GLG1 (amino acid hydrolysis).

Major fractions of the low-dose genes associated with homeostasis genes (Table S4) were associated with stress response (56 genes) and cancer-related functions (43 genes). The low-dose genes were assigned to diverse signal transduction pathways (Table S5) including cell cycle, survival and apoptosis (17 genes), DNA repair, recombination and replication (26 genes), as well as a variety of immune response and cell-signaling (52 genes). Overall, many of these transcripts were associated with or interacted with intercellular-membrane bound organelles and involved in membrane transport.

As illustrated in our working model of low-dose damage response pathways (Figure 3), the signal transduction pathway genes implicate effectors of several major network nodes (e.g., EGR2, TP53, MYC, FOS), that include the p38 MAPK, SAPK/JNK, JAK/STAT, JAK/AKT, cytokine IL2, and IL4, and NF- κ B signaling. The Janus kinase/signal transducers and activators of transcription (JAK/STAT), mitogen-activated protein kinases (MAPK/p38; MEKK), extracellular signal regulated kinases (ERK), phosphatidyl-inositol-3-kinase (PI3/AKT), stress activated protein kinases (SAPK/JNK), IL4, camp, and TGFB intracellular signaling pathways are cascades that transduce signals for homeostasis, stress, inflammatory response and apoptosis. The activating signals include cell surface receptors like cytokines, lymphokines and growth factors that signal through superfamilies of serine and threonine kinases that are known to be IR activated [23]. The cellular balance of these pathways could play a role in determining the cytoprotective versus apoptotic outcomes [54,55] and remains to be fully understood.

Gene-interaction networks and nodal genes

Analyses of the “candidate” set of 291 genes identified 9 major significant low-dose gene-interaction networks with p-value range from 10^{-20} to 10^{-53} (Figure 4). These networks consisted of 279 gene associations, and diverse top functions including cell-cell signaling interaction, heat shock/oxidative stress, transcriptional regulation, cell cycle regulation, kinase signaling, translation regulation and fatty acid metabolism. The 9 networks were merged to create an integrated gene-interaction network with three major hubs: MYC, FOS and TP53 (i.e., genes with >8 interactions with other genes whose expression was modulated in our study). All the sub networks have at least an overlap with the other networks via one or more genes except networks 7 and 8 (by Ingenuity). Furthermore, the IHOP knowledge data base identified connections

among all networks of Figure 4. Evaluations of the 81-gene consistent set identified two networks (scores: 10^{-22} and 10^{-24}), broadly associated with maintenance of cellular homeostasis, signal transduction pathways and immune response (Table S5). The top pathways and functions significantly enriched in the merged MYC-TP53-FOS networks are listed in Table 4.

Conservation of networks and nodes among diverse low dose transcript databases

To determine how well our findings from HL cell lines relate to low dose radiation responses in other cell types, we investigated four human and mouse low dose transcript databases for conserved networks and nodes (see Pathway and Network methods section for details). We identified 5 significant networks for the HUVECs with network score range of 10^{-53} - 10^{-15} , including networks containing MYC, MYCN, TP53 and FOS nodes. The top functions associated with these HUVEC networks are protein synthesis, cellular growth and development, cell cycle and nucleic acid metabolism. Analyses for primary human keratinocytes identified 4 major networks with scores of 10^{-22} - 10^{-15} , containing the nodes MYC, MYCN, TP53 and FOS. The top functions of these networks were associated with gene expression, cell to cell signaling and interaction, cellular assembly and organization. For the mouse brain data, we identified six networks, with SRC, TP53, MYC and FFOS as major nodes. As summarized in Table 5, we found interactive gene networks with MYC, TP53, and FOS nodes in all four cell type radiation response databases. Taken together these data suggest that functions associated with these networks and nodal genes are conserved in low dose damage responses across these different cell systems and species (mouse and human). Furthermore, the data also suggest that these low dose radiation responses are independent of cycling status (lymphoblastoid cells were irradiated in log phase, skin and endothelial cells were grown to near confluence before irradiation, and mouse

brain is a non-cycling tissue in vivo). However, as shown in Table 6, in irradiated lymphocyte cells, we also found certain low-dose radiation-responsive genes that were associated with distinct lymphocyte-specific functions, suggesting that low dose exposures are likely to also induce numerous cell-type specific responses.

Discussion:

The limitations of epidemiological extrapolations to low-dose exposures continue to fuel the scientific and public-policy debates on how to estimate health effects for low-dose exposures [5,7]. There is a major question as to whether low-dose tissue responses are linear as proposed by the LNT model, super-linear with higher relative risks at low doses, or sublinear/threshold with correspondingly lower risks at low doses. We report that exposure of two HL cell lines from unrelated individuals to low-LET ionizing radiation in the range of 1-10 cGy modulated the transcript levels of ~ 80 genes in a consistent manner in both cell lines. We employed design features that enhanced our ability to identify low-dose radiation responsive genes: (i) 6 doses including sham were tested in triplicate; and (ii) results from two independent cell lines were used to generate a permissive genes set (“candidate set”) based on minimal evidence for radiation sensitivity as well as a restrictive gene set based on consistent responses across doses and cell lines (“consistent set”). Using bioinformatics tools and knowledgebases, we identified functional attributes related to homeostasis and signal transduction and showed that genes associated with TP53-MYC-FOS nodes were part of a conserved low dose response across tissues and species that appear to be independent of growth status of the cells. The products of modulated low dose genes are found in nuclear and cytoplasmic locations, with a preponderance of membrane associations.

Shape of the transcript dose response curves

We evaluated transcript changes across 5 doses (1-10 cGy) plus sham controls in cell lines from two unrelated individuals. Most modulated genes showed significant overall plateau-like elevations in transcript levels across this dose range (only one showed a reduction). None of the

modulated genes showed a significant negative slope in both cell lines, and only one, thioredoxin domain containing protein 13 (DJ971N18.2), had a significant positive slope in both cell lines (Table 1. Figures 1 and 2). Induction of related antioxidants are known to occur in response to oxidative stress associated with ionizing radiation [56]. Among the modulated genes, 16 genes had significant Y-intercepts in both independent cell lines based on linear regression analyses that excluded the sham group (as shown in Table 2); this finding provides strong suggestive evidence that transcription of these genes was induced at doses lower than 1 cGy. Our finding of significant transcript modulation changes induced by 10 cGy is consistent with prior findings [14,33-37,53]. Also consistent with our findings, Jin and colleagues [38] reported non-linear transcription responses after low dose exposures. Non-linear dose-response relationships have previously been reported at higher doses for non-genomic endpoints, such as chromosome aberrations [57] and cell killing [58] after exposures to low-LET radiation, and more recently in a more limited study of low dose effects on transcript levels [35,37].

Low-dose functions and networks associated with membranes, organelles, and subcellular locations.

Our analysis shows that low-dose radiation modulated the expression of genes associated with membrane functions, intracellular organelles, subcellular structure, and diverse signaling pathways. Genes associated with membrane function were a dominant group and include gene products associated with cellular and nuclear membranes, mitochondria, golgi, ER and lysosomes (Tables 3 and S3). Forty-one of the 81-gene set are associated with intercellular-membrane bound organelles. Fifty-nine are known to interact with membranes. Our analyses also identified genes encoding small molecule membrane transport proteins (e.g., the SLC family,

TFRC etc), which play roles in maintaining cellular homeostasis and responding to cellular stress. High doses of radiation are known to induce changes in sialic acid lectin-, and calcium-binding membrane sites that lead to the decrease of negative charge in several cell types [59,60]. Our findings suggest that similar mechanisms may function after low doses exposures. We also identified transport molecules and a large number of ion channels such as the solute carrier family of proteins (see Results) that facilitate the passage of selected solutes across the lipid barrier [61-63]. Our findings are consistent with those of Mercier and colleagues who found membrane-bound and mitochondrial proteins to be differentially expressed after low dose exposures in yeast [64].

Our analysis implicates diverse signaling pathways and cytoskeleton maintenance functions. Previous studies showed that high doses of ionizing radiation can alter cell shape, cell surface micromorphology and subcellular organelles [65,66]. Annexins (ANX family) are members of a multigenic family of Ca^{2+} -dependent phospholipid-, membrane- and cytoskeleton-binding proteins that have been implicated in the regulation of the inflammatory response, the structural organization of membranes, ion flux across membranes for signaling and for disease [67]. We identified transcripts encoding CD48, CD58 and CD164, which are membrane bound receptors and cell membrane glycoproteins that participate in cellular–organelle interactions [68]. Our findings predict that exposure to low-dose ionizing radiation leads to biochemical changes in subcellular membranes, but it is not known whether these are direct effects of ionizing radiation or secondary events of the repair response. High resolution imaging of changes in membrane structure and function after low-dose exposure may be a useful next step to further our understanding of low-dose signaling networks and to differentiate tissue- and cell-specific damage response pathways.

Most of what we know about nodal genes of these networks is related to their functions in cancer biology, not low-dose radiation biology. Only a few prior low-dose radiation papers have implicated these pathways in modulating low dose radiation outcomes [15,69]. Our gene interaction model (Figure 4) identifies genes with a high degree of interaction among 9 major nodes, including TP53, MYC and FOS, and can help elucidate the low-dose radiation effects.

TP53 network. TP53 is a complex and multifunctional transcription factor known to respond to a broad variety of cellular stresses, including DNA damage, mitotic spindle damage, heat shock, metabolic changes, hypoxia, viral infection, and oncogene activation [70]. Our analyses found several interesting genes in the TP53 network: the Far upstream element-binding protein (FUBP1), known to regulate MYC proto-oncogene expression, the cytokine family with sequence similarity 3 member C (FAM3C), numerous proteolytic and metabolic genes (LIPA; CTSZ; P4HA1 and SF3B1), RANBP2 a small GTP-binding protein of the RAS superfamily and RAD54B a member of the SNF2/SWI2 superfamily that is involved in the recombinational repair of DNA damage. We identified a direct link between TP53 and the GRP58 node through HSP8. GRP58 is a chaperone glucose-regulated protein associated with stress response and was previously associated with radiation resistance and low-dose effects [36,71]. Thus, TP53 may direct or control low dose stress responses through heat shock protein functions. In the future, analysis of the subcellular localization of TP53-associated gene products after low-dose exposures may help us to better understand the role of the TP53-associated genes.

MYC network. Our analyses also implicated MYC as a major node in the low-dose response, and

c-MYC has been previously found to have a low-dose effect [69]. Eleven genes were directly associated with the MYC node. Many of these genes are known oncogenes. Our low-dose gene set included a strong apoptosis-associated gene, TNF receptor form 6 (TNFRSF6), that was previously associated with high-dose transcript response [20,72] for differing qualities of ionizing radiation [53]. Interestingly, we also identified two low-dose responsive genes thought to be associated with anti-apoptotic functions: CD83 and PTMA. How these pro- and anti-apoptotic genes interact to determine cell fate after low doses remains unknown. The low-dose MYC network also contained several membrane bound genes involved in cytoskeletal organization, metabolism, and immune-related functions: Peptidylglycine alpha-amidating monooxygenase (PAM) a multifunctional metabolism protein; Golgi sialoglyco protein (GLG1) a conserved membrane sialoglycoprotein found within the Golgi of most cells; Tumor differentially expressed 1 (TDE1) which is overexpressed in lung tumors [73]; Lysosomal membrane glycoprotein (LAMP2) that is responsible for the degradation processes; Cap protein (CAPZB, also known as Actin beta) an actin-binding protein and Dead/h box17 (DDX17) a DEAD box (asp-glu-ala-asp/his) RNA helicase that may alter protein-RNA interactions as a splicing regulatory factor [74]. Several of the genes of the MYC node have immune/lymphocyte functions: ROCK1, a downstream effector of Rho, involved in remodeling of the actin cytoskeleton; CD48 an activation-associated cell surface glycoprotein expressed primarily in mitogen-stimulated human lymphocytes; TNFRSF6 (also known as CD95 or FAS) is a oncogene that is dependent on cell surface interactions and functional MYC to induce apoptosis [75]; Prothymosin-alpha (PTMA) important for immune function and possibly anti-apoptotic functions [76]; Activated leukocyte cell adhesion molecule (ALCAM also known as CD6) is a receptor implicated in cell adhesion interactions. Other indirectly associated genes include CD83

an immune-related adhesion receptor; the interferon-induced protein (G1P2) and Gamma-glutamyl hydrolase (GGH) a metabolic enzyme. Our analysis also implicates N-MYC, a member of the MYC family in the low dose response. N-MYC is a primary regulator of cell growth by stimulating genes functioning in ribosome biogenesis and protein synthesis, however, dysregulated N-MYC functions as an oncogene in stem cells.

FOS network. The FOS transcription factor family is radiation-sensitive at high doses and controls diverse functions such as proliferation, differentiation, transformation and even apoptosis [77]. The FOS oncogene has previously been identified among low dose effects [69,78]. Major genes associated with the low-dose FOS network (Figure 4) were: Reticulocalbin 1 (RCN1) a calcium-binding protein located in the ER lumen; Hyaluronan-mediated motility receptor (HMMR) which interacts with RHAMM to respond to wound healing [79]; Tumor rejection antigen 1 (TRA1) a protective gene linked to chaperonins and cytokines IL12 and TNFA; Secretory carrier membrane protein 1 (SCAMP1) a putative transmembrane / leucine zipper-like containing protein and CD164 a sialomucin membrane-associated protein that can be cytoprotective. The FOS-associated genes seem to be primarily associated in cytoprotective effects, e.g., CD164, TRA1, and HMMR1. Others have noted the protective effects of inducing proteins such as IL12, which is downstream of TRA1. The FOS pathway is also directly associated with genes in the network with the early growth response gene-2 (EGR2) nodal gene, which is co-regulated with EGR1. EGR2 suppressed the growth of cancer cells significantly [80] and might mediate the growth suppressive effect of the protein called phosphatase and tensin homology (PTEN) [80].

Comparison of results with other low-dose transcript data sets

We compared our radiation-responsive genes (Table1 and Table S1) with findings of other low-dose transcript studies [13,15,33-39,41]. Using our 81-gene set, elevated GGH transcript was in common with the mouse brain findings [19], TNFRSF6 with ML-1 cells [13], SCAMP1 with keratinocytes [34] and RPL5 with HUVE cells [36]. For the 291 set, our study had 8 and 7 genes in common with keratinocytes and HUVE cells, respectively. Eleven genes were in common between the keratinocytes and HUVE cell studies. Our study also had 9 genes in common with the findings of our adaptive response study [15]. Long et al [37] examined the early X-ray radiation response of human lymphoblastoid AHH-1 cells and reported the induction of genes associated with signal transduction pathways at 4 hours after 5 cGy exposures. Jin et al [38] did a more extensive study in B10 stem cells where they reported distinct clusters in the early response category (1-4 hr) that exhibited dose-, time-, and dose-time dependent responses after 10-50 cGy of gamma radiation. Our lymphoblastoid cell study supports their conclusions that the global transcription response to low doses of gamma radiation appears to follow a non-linear threshold response that involves multiple genes.

Mechanisms and implications

Our current findings suggest that after low-dose exposures, cells recruit homeostasis genes associated with broad aspects of cellular metabolism, as well as stress response genes that seem to be different from those typically induced after high dose exposures. However, transcripts commonly seen after high doses and associated with cell cycle arrest (e.g., CDKN1A and DDB2) and apoptosis (CASP genes) were not seen specifically in our low-dose only study. Although our study design does not allow us to differentiate radiation-specific responses from general stress responses, we did detect elevated levels of DNA damage response transcripts, such

as CDKN1A, RAD54, and GADD45A. Many of the low-dose genes identified had membrane associations and the major gene networks contained large numbers of genes with immune functions. Our findings support the growing evidence that heat shock, transcription and cell cycle responses are transcriptionally modulated after low doses of radiation. The consistency of finding the TP53, MYC and FOS nodes across human and mouse tissues (Table 5) and their broadly associated functions (Table 4) suggest that there are significantly conserved biological response functions after low dose exposures. In a recent study, we have also shown a strong concordance between signaling pathways that are down regulated in the mouse brain and those that are down regulated with normal human aging and in patients with Alzheimer's diseases [81]. Similar effects have been seen in vivo using human skin [41]. An interesting finding of our present study is that low-dose exposures induce a substantial number of genes that have been associated with carcinogenesis and metastasis, but whether their roles are protective or detrimental to cancer induction is unknown at this time; many of the genes involved are known for their protective functions. Our findings underscore the complexity of the dose response relationship for low-dose sensitive genes and illustrate the wide spectrum of the biochemical processes affected by low-dose radiation in multiple cell types. Our findings also strengthen the weight of evidence against using the LNT model for extrapolating health risks to low-dose exposures until we know more about the molecular mechanisms of the low-dose damage response in radiation-sensitive tissues.

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Figure Legends

Figure 1. RT-PCR validation of microarray results

The quantitative RT–PCR values (black circles) and microarray data (gray circles) are represented as means with 95% confidence intervals across the low dose range (1-10 cGy).

Genes tested were: *CD164* (immune modulation), *GGH* (post-translational processing), *LIPA* (lipid metabolism), *PPT1* (protein-lipid metabolism), *SCAMP1* (intracellular protein trafficking), *SSR1* (membrane traffic), *PAM* (oxidoreductase activity), *TMP21* (transmembrane traffic), and *ACSL3* (acyl-coa synthetase long chain family member 3). All genes were normalized to *GAPDH* levels within each cell line; the results did not change notably when two other control genes were used (i.e., beta actin or ribosomal gene). The dotted line represents no radiation effect (i.e., unity fold change between irradiated and control samples).

Figure 2. Dose-response relationships for example genes in two cell lines. The fold change values of transcript response vs. dose (cGy) plotted for cell lines GM15036 (left panels) and GM15510 (right panels). Genes *GGH* and *HNRPD* are representative examples of genes with significant Y intercepts with non-significant slopes in both cell lines (this was the most common dose-response observed in our study). Triplicate data are shown at each dose. Black lines represent the linear regression lines; gray boxes represent the value range in unirradiated samples.

Figure 3. Signaling pathways and subcellular location model for low-dose radiation response. Genes listed in parenthesis were transcriptionally modulated on microarrays or identified in networks (Figure 4), as evidence for the involvement of pathways and functions.

Figure 4. Composite model of gene interaction networks after low-dose cellular exposures.

A composite model of gene networks with the 291 low dose (1-10 cGy) IR responsive gene set (p-value range from 10^{-20} to 10^{-53}). Genes differentially expressed according to the microarray findings are shown in red. Networks include genes that were not detected in our experiment but that are known to have physical or functional associations with the genes differentially expressed after low dose. Networks with common genes are connected by bold lines.

Table 1. Set of 81 probe sets with consistent low-dose response (1-10cGy) in cell lines derived from two unrelated individuals (in alphabetical order; 75 unique genes represented)

	GENE SYMBOL	ACCESSTION NUMBER	Line GM15036				Line GM15510			
			Fold-change ^a	CI-low ^b	CI-high ^b	p-value ^c	Fold-change ^a	CI-low ^b	CI-high ^b	p-value ^c
1	AF311304	NM_031214	2.0	1.7	2.4	0.048	2.2	1.8	2.7	0.003
2	ALG5	NM_013338	1.8	1.5	2.0	0.001	1.8	1.6	1.9	0.000
3	ATP10D	AI478147	2.3	1.7	3.0	0.018	2.2	1.8	2.5	0.004
4	ATP1B3	U51478	1.5	1.3	1.7	0.000	1.5	1.3	1.7	0.000
5	BTA1F1	AJ001017	1.7	1.4	1.9	0.020	1.4	1.3	1.5	0.019
6	C11orf15	NM_020644	1.6	1.5	1.8	0.010	1.5	1.4	1.6	0.004
7	C1orf8	NM_004872	2.1	1.6	2.6	0.011	1.6	1.5	1.7	0.003
8	CD164	NM_006016	1.7	1.4	2.1	0.029	1.9	1.7	2.0	0.000
9	CD48	NM_001778	1.6	1.4	1.8	0.003	1.5	1.4	1.7	0.036
10	CD53	NM_000560	1.4	1.3	1.6	0.012	1.4	1.3	1.5	0.003
11	CD58	BC005930	2.5	1.8	3.1	0.000	1.9	1.8	2.1	0.000
12	CD58	NM_001779	2.0	1.6	2.5	0.000	1.6	1.4	1.7	0.000
13	CD58	D28586	2.2	1.5	2.9	0.018	1.7	1.5	2.0	0.022
14	CGI-100	AL117354	2.1	1.5	2.7	0.010	1.5	1.4	1.7	0.004
15	CKLFSF6	NM_017801	1.7	1.5	1.9	0.001	1.6	1.4	1.7	0.004
16	CNIH	NM_005776	1.8	1.4	2.2	0.041	1.5	1.4	1.6	0.016
17	COCH	AA669336	1.6	1.4	1.9	0.029	1.5	1.4	1.6	0.013
18	DJ971N18.2	BF572868	2.2	1.7	2.8	0.035	2.0	1.8	2.1	0.002
19	DSG2	BF031829	2.3	1.7	2.9	0.012	1.8	1.7	2.0	0.001
20	ELOVL5	AL136939	2.1	1.7	2.4	0.000	1.7	1.5	1.9	0.000
21	ENTPD1	U87967	1.8	1.4	2.2	0.044	1.9	1.7	2.1	0.000
22	EPRS	NM_004446	1.6	1.3	1.9	0.044	1.4	1.3	1.5	0.005
23	FACL3	AL525798	1.6	1.4	1.7	0.048	1.6	1.5	1.7	0.007
24	FLJ10652	NM_018169	1.8	1.5	2.1	0.014	1.7	1.4	2.0	0.011
25	FLJ10900	NM_018264	1.7	1.4	1.9	0.042	1.7	1.5	2.0	0.005
26	GALNT7	NM_017423	1.8	1.4	2.2	0.042	1.8	1.7	2.0	0.001
27	GGH	NM_003878	2.5	1.8	3.2	0.001	1.9	1.8	2.1	0.000
28	GLG1	AK025457	1.8	1.5	2.2	0.025	1.9	1.7	2.1	0.000
29	GMH1	NM_014044	2.4	2.0	2.8	0.000	1.8	1.6	2.0	0.001
30	HIP14	AI621223	1.7	1.6	1.9	0.001	1.5	1.4	1.7	0.004
31	HLA-B	D83043	1.7	1.4	1.9	0.010	1.5	1.3	1.7	0.011
32	HMGCR	AL518627	2.1	1.7	2.5	0.011	1.8	1.5	2.1	0.047
33	HMMR	NM_012485	2.0	1.6	2.3	0.047	1.6	1.4	1.7	0.036
34	HNRPD	W74620	3.1	2.4	3.8	0.000	2.0	1.8	2.3	0.000
35	HTGN29	NM_020199	1.7	1.4	2.0	0.014	1.5	1.4	1.6	0.002
36	IFI44	BE049439	2.2	1.7	2.6	0.016	1.9	1.7	2.1	0.002
37	JWA	NM_006407	1.7	1.4	1.9	0.006	1.6	1.5	1.7	0.001
38	KIAA0102	BF530535	2.1	1.7	2.4	0.000	1.6	1.6	1.7	0.000
39	KTN1	Z22551	1.5	1.4	1.7	0.043	1.6	1.4	1.8	0.023
40	LIPA	NM_000235	2.2	1.7	2.7	0.001	2.0	1.9	2.2	0.000

41	LOC56851	NM_020154	2.2	1.6	2.7	0.000	1.8	1.6	2.0	0.000
42	LYRIC	AI972475	1.4	1.3	1.5	0.012	1.3	1.2	1.5	0.049
43	MAN1A1	BG287153	2.0	1.5	2.5	0.033	1.8	1.5	2.1	0.036
44	MGC5306	NM_024116	2.1	1.7	2.5	0.006	2.0	1.8	2.2	0.000
45	MGC8721	NM_016127	2.0	1.7	2.2	0.000	1.6	1.4	1.7	0.001
46	MS4A1	BC002807	1.7	1.4	2.0	0.025	1.5	1.4	1.7	0.037
47	P5	AK026926	2.1	1.7	2.5	0.000	1.6	1.3	1.8	0.001
48	P5	NM_005742	1.5	1.3	1.6	0.013	1.5	1.3	1.7	0.013
49	P5	BC001312	1.5	1.3	1.6	0.018	1.4	1.3	1.6	0.011
50	PAM	NM_000919	1.5	1.4	1.7	0.044	1.5	1.4	1.6	0.008
51	POLQ	NM_014125	1.9	1.6	2.2	0.039	1.6	1.6	1.6	0.005
52	PRDX4	NM_006406	1.8	1.5	2.1	0.004	1.9	1.6	2.1	0.000
53	PTPRC	Y00062	2.3	1.8	2.7	0.010	1.7	1.6	1.9	0.013
54	PTTG1IP	NM_004339	1.6	1.4	1.9	0.003	1.4	1.3	1.5	0.004
55	RNASET2	NM_003730	1.7	1.5	1.9	0.010	1.6	1.4	1.7	0.005
56	RNASET2	NM_003730	1.4	1.3	1.5	0.047	1.5	1.4	1.6	0.003
57	RPL5	AL137958	1.9	1.4	2.3	0.034	1.8	1.7	1.9	0.000
58	RPN2	BC003560	1.8	1.5	2.2	0.013	1.6	1.5	1.7	0.001
59	SART2	NM_013352	1.9	1.7	2.2	0.002	1.8	1.6	2.0	0.001
60	SCAMP1	AV745949	1.8	1.5	2.1	0.015	2.0	1.8	2.3	0.000
61	SLC1A1	AW235061	1.8	1.4	2.1	0.011	1.7	1.6	1.8	0.001
62	SLC30A1	AI972416	2.0	1.6	2.4	0.004	2.0	1.7	2.3	0.000
63	SLC35A3	NM_012243	1.8	1.5	2.1	0.046	1.9	1.6	2.1	0.028
64	SLC38A1	NM_030674	1.8	1.6	2.1	0.001	1.5	1.4	1.6	0.005
65	SLC39A6	AI635449	1.9	1.6	2.2	0.001	1.8	1.6	2.1	0.000
66	SMBP	NM_020123	1.6	1.4	1.8	0.043	1.6	1.4	1.8	0.010
67	SORL1	AV728268	1.7	1.5	2.0	0.009	1.6	1.5	1.8	0.001
68	SPTLC1	AL568804	2.1	1.7	2.5	0.011	2.0	1.8	2.3	0.003
69	SQLE	AF098865	2.0	1.6	2.4	0.021	1.9	1.5	2.2	0.015
70	SSR1	AI016620	1.7	1.4	2.1	0.006	2.0	1.7	2.2	0.000
71	TEB4	BF000409	1.5	1.4	1.6	0.005	1.6	1.4	1.8	0.001
72	TFRC	NM_003234	2.6	1.9	3.2	0.000	1.6	1.4	1.7	0.000
73	TGOLN2	W72053	1.5	1.4	1.6	0.037	1.4	1.3	1.5	0.015
74	TLOC1	U93239	1.8	1.5	2.0	0.000	1.6	1.4	1.8	0.000
75	TM9SF2	NM_004800	2.6	1.8	3.3	0.002	2.2	1.9	2.4	0.000
76	TMP21	BE780075	1.9	1.5	2.3	0.020	1.9	1.6	2.1	0.001
77	TNFRSF6	AA164751	2.0	1.5	2.6	0.003	1.8	1.5	2.0	0.009
78	TRAM1	BC000687	1.8	1.4	2.2	0.015	2.0	1.9	2.1	0.000
79	TXNDC4	BC005374	2.5	1.9	3.1	0.000	2.1	1.8	2.4	0.000
80	VMP1	NM_030938	1.7	1.4	1.9	0.000	1.4	1.3	1.5	0.000
81	ZMPSTE24	NM_005857	1.8	1.4	2.1	0.010	1.7	1.6	1.9	0.000

- a) Average of fold change across 5 low doses (1-10cGy) each with three technical replicates (i.e., 15 values)
b) Upper and lower Confidence interval (95 %) around the average fold change
c) FDR (False Discovery Rate) adjusted p value for multiple comparisons.

Table 2. Genes with a significant Y-intercept in both cell lines from two unrelated individuals, suggesting radiation sensitivity below 1 cGy.

Genes	GM15036						GM15510					
	SHAM ^a		Low Dose Irradiation ^b				SHAM ^a		Low Dose Irradiation ^b			
	CI-low	CI-high	Intercept	P-value	CI-low	CI-high	CI-low	CI-high	Intercept	P-value	CI-low	CI-high
P5	0.98	1.02	2.38	3.78E-13	1.79	2.97	0.90	1.10	2.65	1.33E-13	2.05	3.25
SLC30A1	0.94	1.06	2.32	5.29E-15	1.50	3.14	0.87	1.13	2.44	2.66E-15	1.58	3.31
ATP10D	0.96	1.04	2.81	1.09E-13	2.16	3.46	0.88	1.12	2.40	3.64E-14	1.73	3.06
SCAMP1	0.91	1.09	2.07	3.04E-15	1.20	2.93	0.88	1.12	2.35	3.76E-13	1.79	2.91
DSG2	0.86	1.14	2.54	4.34E-16	1.46	3.61	0.86	1.14	2.35	4.85E-13	1.80	2.89
TM9SF2	0.91	1.09	3.01	1.31E-14	2.25	3.77	0.95	1.05	2.26	1.80E-14	1.56	2.96
SQLE	0.90	1.10	2.44	2.58E-15	1.57	3.32	0.93	1.07	2.17	5.05E-14	1.52	2.82
DJ971N18.2	0.78	1.22	2.69	6.48E-13	2.12	3.25	0.88	1.12	2.16	1.53E-13	1.57	2.76
HMGCR	0.94	1.06	2.41	1.71E-13	1.79	3.04	0.96	1.04	2.12	1.75E-11	1.69	2.55
HNRPD	0.93	1.07	3.52	4.21E-15	2.68	4.36	0.88	1.12	2.10	6.05E-15	1.32	2.88
GGH	0.92	1.08	2.98	6.91E-16	1.99	3.96	0.90	1.10	2.07	4.89E-15	1.27	2.87
GALNT7	0.90	1.10	2.16	1.30E-14	1.40	2.92	0.90	1.10	2.06	1.78E-14	1.35	2.76
SPTLC1	0.84	1.16	2.12	2.22E-14	1.39	2.85	0.70	1.30	2.04	4.80E-14	1.39	2.69
TMP21	0.87	1.13	2.15	1.51E-15	1.24	3.06	0.90	1.10	1.86	6.00E-14	1.22	2.49
CD58	0.92	1.08	2.33	4.78E-16	1.27	3.40	0.90	1.10	1.72	1.19E-11	1.28	2.16
MAN1A1	0.94	1.06	2.21	8.70E-13	1.65	2.76	0.89	1.11	1.72	1.18E-12	1.20	2.23

- (a) Each of the triplicate values of log2 expression were normalized with reference to the average value and 95% confidence level was calculated for sham exposures.
- (b) Linear regression analyses were performed on the dose response of the fold change. P-value: FDR adjusted p-value for multiple comparisons. Confidence Interval of the intercept calculated at 95%.

Table 3. Comparison of Gene Ontology (GO) assignments of low dose genes to biological process and cellular location for the candidate 291-gene and consistent 81-gene sets.

Category	Terms	Candidate 291-gene set			Consistent 81-gene set		
		Count (a)	% (b)	p-value (c)	Count (a)	% (b)	p-value (c)
Biological Process	Cellular homeostasis	16	6	8.7E-5	6	7.8	1.5E-2
	Establishment of protein localization	21	7.9	4.7E-3	8	10.4	6.0E-2
	Cation transport	15	5.7	7.6E-3	6	7.8	8.1E-2
	Response to unfolded protein	6	2.3	7.8E-3			
	Protein transport	19	7.2	9.7E-3	8	10.4	4.4E-2
	Cellular ion homeostasis	10	3.8	1.2E-2			
	Glycoprotein biosynthetic process				5	6.5	3.5E-3
	Amino acid transport				3	3.9	2.9E-2
Cellular Location	Endomembrane system	57	21.5	5.6E-17	26	33.8	3.8E-11
	Endoplasmic reticulum	51	19.2	6.7E-16	23	29.9	4.0E-10
	Integral to membrane	114	43	2.3E-13	58	75.3	4.3E-17
	Membrane part	125	47.2	4.5E-12	59	76.6	2.1E-14
	Cytoplasm	131	49.4	9.5E-5	46	59.7	8.4E-3
	Intracellular organelle	150	56.6	3.0E-4	33	42.9	1.1E-3
	Vacuole	13	4.9	9.9E-4	-	-	-
	Golgi apparatus	21	7.9	7.0E-3	11	14.3	3.9E-3

a. Count: Number of genes in the gene set that are represented in the GO category (level 4 analysis)

b. Percentage representation of the gene set in the GO category

c. P value as determined by EASE

Table 4. Pathways and component genes associated with the MYC-TP53-FOS networks in HL cells

Pathway	<u>-Log 10(P-value)</u>	Component Genes
Aminoacyl-tRNA Biosynthesis	4.7	EPRS, QARS, IARS (includes EG:3376), LARS, RARS, MARS (includes EG:4141)
ERK/MAPK Signaling	2.9	MAPK3, MYC, YWHAZ, FOS, PRKCD, ATF1, MYCN, ETS1
Cell Cycle: G1/S Checkpoint Regulation	2.8	TGFB2,MYC,ATR,CCND2,TP53
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	2.5	YWHAZ, ATR, MDM2 (includes EG:4193), TP53
Endoplasmic Reticulum Stress Pathway	2.5	EIF2AK3, XBP1, HSPA5
p38 MAPK Signaling	2.4	MAX, TGFB2, MYC, MAPKAPK2, ATF1
Estrogen Receptor Signaling	2.1	MAPK3, NCOA3, NCOA1, CCNH, DDX5, NCOA2
Protein Ubiquitination Pathway	1.9	PSMC5, PSMC4, SKP2, PSMD6, MDM2 (includes EG:4193), PSMD1, PSMA2, HSPA5
EGF Signaling	1.5	MAPK3, FOS, EGFR
Antigen Presentation Pathway	1.5	B2M, HLA-C, HLA-B

Table 5. Comparison of gene interaction networks containing TP53, MYC, and FOS nodes among three human and one mouse low dose expression datasets

Network Nodal gene	Human Lymphoblastoid cells* (current study)	Human Umbilical Vein Endothelial cells Lanza et al 2005	Primary Human Keratinocytes Franco et al 2005	Mouse Brain Tissue** Jin et al. 2003, Lowe et al 2008
	In vitro	In vitro	In vitro	In vivo
	Logarithmic growth in suspension	Near confluence in 2D culture	Near confluence in 2D culture	Non-dividing tissue
TP53	16**	15	20	16
MYC	19	54	20	14
FOS	6	12	15	12
MYCN	17	9	22	13

*Table entries are scores for the networks centered on the identified nodal genes (see Figure 3).

** After whole body radiation exposures

Table 6. Low dose responsive genes associated with lymphocyte functions*

<i>Gene ID</i>	<i>Function</i>
IFNGR1	Cytokine Receptor
HLA-DRA	Class II Antigen presenter
ILF2	Immune Response
LRBA	Trafficking of vesicles
HLA-B	Class Antigen presenter
LRMP	Developmental regulation
CD53	Signal transduction
CD63	Blood platelet activation factor
SART2	Tumor-rejection antigen
MCP	Inactivation of complement
ETEA	Resistance to apoptosis
CTSS	Degradation of antigenic proteins
MS4A1	B-cell activation
CD47	Signal activation
EB12	EBV Infection of B cells
CD58	Humoral response
ENTPD1	Humoral response
ALCAM	Humoral response
GIP2	Immune response
CD83	Humoral response
CD48	Defense response
CNIH	Immune response
CD164	Signaling
CD59	Signaling
IF144	Response to virus

* selected from the 81-gene consistent set

Figure 1

Figure 2

The low-dose response network

Figure 3

Figure 4

Table S1. Supporting material. Listing of the genes in the “candidate” 291-gene set

NUMBER OF GENES	GENE SYMBOL	GENE NAME	ACCESSION NUMBER
1	RPL24	ribosomal protein L24	NM_000986
2	SLC25A3	solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3	NM_002635
3	ILF2	interleukin enhancer binding factor 2, 45kDa	NM_004515
4	TRA1	tumor rejection antigen (gp96) 1	AI582238
5	TMEM59	chromosome 1 open reading frame 8	NM_004872
6	CD63	CD63 antigen (melanoma 1 antigen)	NM_001780
7	LAPTM4A	lysosomal-associated protein transmembrane 4 alpha	NM_014713
8	PTTG1IP	pituitary tumor-transforming 1 interacting protein	NM_004339
9	KDEL2	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2	NM_006854
10	ARL6IP5	ADP-ribosylation-like factor 6 interacting protein 5	N92494
11	ARL6IP5	ADP-ribosylation-like factor 6 interacting protein 5	NM_006407
12	PTMA	prothymosin, alpha (gene sequence 28)	BF686442
13	HSPA1A	transcriptional intermediary factor 1	NM_005345
14	EPRS	glutamyl-prolyl-tRNA synthetase	NM_004446
15	TMEM66	hypothetical protein MGC8721	NM_016127
16	DNAJA1	DnaJ (Hsp40) homolog, subfamily A, member 1	NM_001539
17	SSR1	signal sequence receptor, alpha (translocon-associated protein alpha)	AI016620
18	SSR1	signal sequence receptor, alpha (translocon-associated protein alpha)	AW006345
19	PPT1	palmitoyl-protein thioesterase 1 (ceroid-lipofuscinosis, neuronal 1, infantile)	NM_000310
20	CD59	CD59 antigen p18-20 (antigen identified by monoclonal antibodies 16.3A5, EJ16, EJ30, EL32 and G344)	NM_000611
21	PAICS	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase	NM_006452
22	STOM	stomatin	M81635
23	RCN1	reticulocalbin 1, EF-hand calcium binding domain	NM_002901
24	SF3B1	splicing factor 3b, subunit 1, 155kDa	NM_012433
25	TM9SF2	transmembrane 9 superfamily member 2	NM_004800
26	RPS29	ribosomal protein S29	NM_001032
27	CSE1L	CSE1 chromosome segregation 1-like (yeast)	NM_001316
28	PGRMC1	progesterone receptor membrane component 1	NM_006667
29	MBNL1	muscleblind-like (Drosophila)	N31913
30	SPCS2	signal peptidase complex subunit 2 homolog (S. cerevisiae)	BF530535

31	SPCS2	signal peptidase complex subunit 2 homolog (S. cerevisiae)	NM_014752
32	SYPL1	synaptophysin-like protein	NM_006754
33	RARS	arginyl-tRNA synthetase	NM_002887
34	GTF3A	general transcription factor IIIA	NM_002097
35	COPB	coatamer protein complex, subunit beta	NM_016451
36	DHX15	DEAH (Asp-Glu-Ala-His) box polypeptide 15	AF279891
37	TRAM1	translocation associated membrane protein 1	BC000687
38	ATP6AP2	ATPase, H ⁺ transporting, lysosomal accessory protein 2	AF248966
39	RCN2	reticulocalbin 2, EF-hand calcium binding domain	BC004892
40	RCN2	reticulocalbin 2, EF-hand calcium binding domain	NM_002902
41	TXNDC13	hypothetical protein DJ971N18.2	BF572868
42	CNIH	cornichon homolog (Drosophila)	NM_005776
43	ACSL3	acyl-CoA synthetase long-chain family member 3	AL525798
44	ACSL3	acyl-CoA synthetase long-chain family member 3	NM_004457
45	ACSL3	acyl-CoA synthetase long-chain family member 3	D89053
46	SMC4L1	SMC4 structural maintenance of chromosomes 4-like 1 (yeast)	NM_005496
47	RANBP2	RAN binding protein 2	AI681120
48	RANBP2	RAN binding protein 2	NM_006267
49	GALNT1	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1 (GalNAc-T1)	NM_020474
50	6-Mar	membrane-associated RING-CH protein VI	BF000409
51	RNF13	ring finger protein 13	AF070558
52	VDP	vesicle docking protein p115	NM_003715
53	LIPA	lipase A, lysosomal acid, cholesterol esterase (Wolman disease)	NM_000235
54	PRG1	proteoglycan 1, secretory granule	J03223
55	FAM3C	family with sequence similarity 3, member C	NM_014888
56	DCN	signal sequence receptor, alpha (translocon-associated protein alpha)	NM_001920
57	SEC63	SEC63-like (S. cerevisiae)	NM_007214
58	SLC25A36	hypothetical protein FLJ10618	AL049246
59	PRDX4	peroxiredoxin 4	NM_006406
60	HEXB	hexosaminidase B (beta polypeptide)	NM_000521
61	ALCAM	activated leukocyte cell adhesion molecule	AA156721
62	UBE4A	ubiquitination factor E4A (UFD2 homolog, yeast)	NM_004788
63	SLC39A6	solute carrier family 39 (zinc transporter), member 6	AI635449
64	PRPF4B	PRP4 pre-mRNA processing factor 4 homolog B (yeast)	AB011108
65	PCM1	pericentriolar material 1	NM_006197

66	TMED5	transmembrane emp24 protein transport domain containing 5	AL117354
67	SDFR1	stromal cell derived factor receptor 1	NM_017455
68	SLC16A1	solute carrier family 16 (monocarboxylic acid transporters), member 1	NM_003051
69	SPTLC1	serine palmitoyltransferase, long chain base subunit 1	AL568804
70	TAP1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	NM_000593
71	PAM	peptidylglycine alpha-amidating monooxygenase	NM_000919
72	USP1	ubiquitin specific protease 1	NM_003368
73	HMGCR	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	AL518627
74	HMGCR	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	NM_000859
75	STCH	stress 70 protein chaperone, microsome-associated, 60kDa	AI718418
76	ARMET	arginine-rich, mutated in early stage tumors	NM_006010
77	IFNGR1	interferon gamma receptor 1	NM_000416
78	ITM2A	integral membrane protein 2A	NM_004867
79	PSMD6	proteasome regulatory particle subunit p44S10	NM_014814
80	FUCA1	fucosidase, alpha-L- 1, tissue	NM_000147
81	CTSS	cathepsin S	NM_004079
82	ZMPSTE24	zinc metallopeptidase (STE24 homolog, yeast)	NM_005857
83	C5orf15	chromosome 5 open reading frame 15	NM_020199
84	PTPRK	protein tyrosine phosphatase, receptor type, K	NM_002844
85	NDUFS1	NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa (NADH-coenzyme Q reductase)	NM_005006
86	LAMP2	lysosomal-associated membrane protein 2	NM_002294
87	AKAP11	A kinase (PRKA) anchor protein 11	NM_016248
88	SLC33A1	solute carrier family 33 (acetyl-CoA transporter), member 1	NM_004733
89	ABCC4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	AI948503
90	HS2ST1	heparan sulfate 2-O-sulfotransferase 1	AW151887
91	CD53	CD53 antigen	NM_000560
92	GGH	gamma-glutamyl hydrolase (conjugase, folypolygammaglutamyl hydrolase)	NM_003878
93	UNC50	unc-50 homolog (C. elegans)	NM_014044
94	WDR50	isocitrate dehydrogenase 3 (NAD+) gamma	NM_016001
95	SLC9A6	solute carrier family 9 (sodium/hydrogen exchanger), isoform 6	NM_006359
96	CD48	CD48 antigen (B-cell membrane protein)	NM_001778
97	ENPP4	ectonucleotide pyrophosphatase/phosphodiesterase 4 (putative function)	AW194947
98	SMC2L1	SMC2 structural maintenance of chromosomes 2-like 1 (yeast)	NM_006444

99	SLC12A2	solute carrier family 12 (sodium/potassium/chloride transporters), member 2	NM_001046
100	CD83	CD83 antigen (activated B lymphocytes, immunoglobulin superfamily)	NM_004233
101	LRMP	lymphoid-restricted membrane protein	NM_006152
102	SGCE	sarcoglycan, epsilon	NM_003919
103	TGFBR3	transforming growth factor, beta receptor III (betaglycan, 300kDa)	NM_003243
104	FAS	Fas (TNF receptor superfamily member)	AA164751
105	FAS	Fas (TNF receptor superfamily member)	NM_000043
106	SGCB	sarcoglycan, beta (43kDa dystrophin-associated glycoprotein)	U29586
107	CD58	CD58 antigen, (lymphocyte function-associated antigen 3)	NM_001779
108	COCH	coagulation factor C homolog, cochlin (Limulus polyphemus)	AA669336
109	EBI2	Epstein-Barr virus induced gene 2 (lymphocyte-specific G protein-coupled receptor)	NM_004951
110	H2AFX	H2A histone family, member X	NM_002105
111	G1P2	interferon, alpha-inducible protein (clone IFI-15K)	NM_005101
112	STEAP1	six transmembrane epithelial antigen of the prostate	NM_012449
113	CD38	CD38 antigen (p45)	NM_001775
114	ATP6V0A2	ATPase, H ⁺ transporting, lysosomal V0 subunit a isoform 2	NM_012463
115	HMGA1	high mobility group AT-hook 1	NM_002131
116	PSF1	KIAA0186 gene product	NM_021067
117	SLC35A3	solute carrier family 35 (UDP-N-acetylglucosamine (UDP-GlcNAc) transporter), member A3	NM_012243
118	MAPK6	mitogen-activated protein kinase 6	NM_002748
119	HMMR	hyaluronan-mediated motility receptor (RHAMM)	NM_012485
120	ACSL1	acyl-CoA synthetase long-chain family member 1	NM_001995
121	TFRC	transferrin receptor (p90, CD71)	NM_003234
122	P4HA1	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I	NM_000917
123	PDIA6	thioredoxin domain containing 7 (protein disulfide isomerase)	NM_005742
124	ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1	NM_001776
125	POLQ	polymerase (DNA directed), theta	NM_014125
126	LAPTM4B	lysosomal associated protein transmembrane 4 beta	NM_018407
127	TXNDC	thioredoxin domain containing	NM_030755
128	HLRC1	hypothetical protein MGC4293	NM_031304

129	CD164	CD164 antigen, sialomucin	NM_006016
130	NA	prothymosin, alpha (gene sequence 28)	NM_016171
131	SRRM2	serine/arginine repetitive matrix 2	AI655799
132	PDIA3	glucose regulated protein, 58kDa	D83485
133	ATP6V1C2	thioredoxin domain containing 7 (protein disulfide isomerase)	BE910010
134	PDIA6	thioredoxin domain containing 7 (protein disulfide isomerase)	BC001312
135	TDE2	tumor differentially expressed 2	AF164794
136	DDOST	dolichyl-diphosphooligosaccharide-protein glycosyltransferase	D29643
137	RPN2	ribophorin II	BC003560
138	HLA-B	major histocompatibility complex, class I, B	D83043
139	MCP	membrane cofactor protein (CD46, trophoblast-lymphocyte cross-reactive antigen)	AL570661
140	ELOVL5	ELOVL family member 5, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)	AL136939
141	ATP1B3	ATPase, Na ⁺ /K ⁺ transporting, beta 3 polypeptide	U51478
142	CANX	calnexin	AI761759
143	EDD1	progesterin-induced protein	AF006010
144	HLA-DRA	major histocompatibility complex, class II, DR alpha	M60334
145	RPS28	ribosomal protein S28	BC000354
146	TLOC1	translocation protein 1	U93239
147	TXNDC4	thioredoxin domain containing 4 (endoplasmic reticulum)	BC005374
148	UCP2	uncoupling protein 2 (mitochondrial, proton carrier)	U94592
149	DLD	dihydrolipoamide dehydrogenase (E3 component of pyruvate dehydrogenase complex, 2-oxo-glutarate complex, branched chain keto acid dehydrogenase complex)	J03620
150	SQLE	squalene epoxidase	AF098865
151	METTL3	methyltransferase like 3	BC001650
152	SLC39A8	solute carrier family 39 (zinc transporter), member 8	AB040120
153	SWAP70	SWAP-70 protein	AB014540
154	MSH2	mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)	U04045
155	BTAF1	BTAF1 RNA polymerase II, B-TFIID transcription factor-associated, 170kDa (Mot1 homolog, S. cerevisiae)	AJ001017
156	ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1	AV717590
157	ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1	U87967
158	RNF139	ring finger protein 139	AF064801
159	MINPP1	multiple inositol polyphosphate histidine phosphatase, 1	AF084943

160	HMMR	hyaluronan-mediated motility receptor (RHAMM)	U29343
161	KIAA0922	KIAA0922 protein	AL136932
162	CTSZ	cathepsin Z	AF073890
163	PHTF1	putative homeodomain transcription factor 1	BC002447
164	HSPA8	heat shock 70kDa protein 8	AB034951
165	MS4A1	membrane-spanning 4-domains, subfamily A, member 1	BC002807
166	C14orf2	chromosome 14 open reading frame 2	AF116639
167	DKFZp564K142	implantation-associated protein	AF130104
168	AKAP9	A kinase (PRKA) anchor protein (yotiao) 9	AB019691
169	HLA-DRA	major histocompatibility complex, class II, DR alpha	M60333
170	CD47	CD47 antigen (Rh-related antigen, integrin-associated signal transducer)	Z25521
171	ATP2C1	ATPase, Ca++ transporting, type 2C, member 1	AF189723
172	SC5DL	sterol-C5-desaturase (ERG3 delta-5-desaturase homolog, fungal)-like	D85181
173	RTN4	reticulon 4	AB015639
174	IFNGR1	interferon gamma receptor 1	AF056979
175	CD58	CD58 antigen, (lymphocyte function-associated antigen 3)	BC005930
176	SLD5	SLD5	BC005995
177	HSPA5	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)	AF216292
178	ITGB1	integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)	BG500301
179	PNN	pinin, desmosome associated protein	AW152664
180	TGOLN2	trans-golgi network protein 2	W72053
181	ETEA	protein expressed in T-cells and eosinophils in atopic dermatitis	AB020694
182	SLC39A14	solute carrier family 39 (zinc transporter), member 14	D31887
183	NIPA2	non imprinted in Prader-Willi/Angelman syndrome 2	AI589507
184	KIDINS220	kinase D-interacting substance of 220 kDa	AB033076
185	DKFZP564G2022	DKFZP564G2022 protein	BG493972
186	DKFZP564G2022	DKFZP564G2022 protein	AL049944
187	MTDH	LYRIC/3D3	AV700332
188	MTDH	LYRIC/3D3	AI972475
189	RPL17	ribosomal protein L17	BG168283
190	PSMD14	proteasome (prosome, macropain) 26S subunit, non-ATPase, 14	NM_005805
191	YIPF6	hypothetical protein MGC21416	AA195936
192	CREB3L2	cAMP responsive element binding protein 3-like 2	BE675139
193	TMED10	transmembrane trafficking protein	BE780075
194	RDX	radixin	AL137751

195	TOR1AIP1	lamina-associated polypeptide 1B	AK023204
196	SCAMP1	secretory carrier membrane protein 1	AV745949
197	LASS6	LAG1 longevity assurance homolog 6 (S. cerevisiae)	BG289001
198	AZIN1	ornithine decarboxylase antizyme inhibitor	BF793951
199	ATP11B	ATPase, Class VI, type 11B	AB023173
200	SORL1	sortilin-related receptor, L(DLR class) A repeats-containing	AV728268
201	PTPRC	protein tyrosine phosphatase, receptor type, C	Y00062
202	ARID5B	AT rich interactive domain 5B (MRF1-like)	BG285011
203	TMEM41B	KIAA0033 protein	N64760
204	TMEM41B	KIAA0033 protein	AU153138
205	JMJD1A	jumonji domain containing 1A	AA524505
206	LRBA	LPS-responsive vesicle trafficking, beach and anchor containing	W60686
207	FTL	ferritin, light polypeptide	BG537190
208	SLC30A1	solute carrier family 30 (zinc transporter), member 1	AI972416
209	DCP2	DCP2 decapping enzyme homolog (S. cerevisiae)	AV715578
210	MRPS6	mitochondrial ribosomal protein S6	AK024896
211	ZDHHC17	zinc finger, DHHC domain containing 17	AI621223
212	ROCK1	Rho-associated, coiled-coil containing protein kinase 1	N22548
213	MRPS6	solute carrier family 5 (inositol transporters), member 3	AI867198
214	CAP350	centrosome-associated protein 350	AI041204
215	ATP10D	ATPase, Class V, type 10D	AI478147
216	HNRPD	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa)	W74620
217	RPL37A	ribosomal protein L37a	AU155515
218	SFRS7	splicing factor, arginine/serine-rich 7, 35kDa	AA524053
219	METTL3	methyltransferase like 3	AW069290
220	SLC1A1	solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1	AW235061
221	FAM69A	hypothetical LOC388650	AL137958
222	DDX17	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	AW188131
223	IFI44	interferon-induced protein 44	BE049439
224	FUBP1	far upstream element (FUSE) binding protein 1	AA156865
225	MATR3	matrin 3	AA129420
226	RTN4	reticulon 4	AF320999
227	KTN1	thioredoxin domain containing 7 (protein disulfide isomerase)	Z22551
228	GLG1	kinectin 1 (kinesin receptor)	AK025457
229	GTF3A	golgi apparatus protein 1	BE542815
230	LOC348162	general transcription factor IIIA	AL049250
231	FAM69A	PI-3-kinase-related kinase SMG-1-like	AK027146
232	B2M	hypothetical LOC388650	AW188940

233	LOC440085	beta-2-microglobulin	AF257099
234	CYP51P2	prothymosin, alpha (gene sequence 28)	U40053
235	ITPR2	cytochrome P450, family 51, subfamily A, polypeptide 1	AL049988
236	PDIA6	thioredoxin domain containing 7 (protein disulfide isomerase)	AK026926
237	CD58	CD58 antigen, (lymphocyte function-associated antigen 3)	D28586
238	ITM2B	integral membrane protein 2B	AF092128
239	TMSB10	thymosin, beta 10	NM_021103
240	TMEM30A	transmembrane protein 30A	NM_018247
241	SMBP	endomembrane protein emp70 precursor isolog	NM_020123
242	GOLPH2	golgi phosphoprotein 2	NM_016548
243	ARL10C	ADP-ribosylation factor-like 10C	NM_018184
244	HSD17B12	hydroxysteroid (17-beta) dehydrogenase 12	NM_016142
245	C15orf24	chromosome 15 open reading frame 24	NM_020154
246	DSG2	desmoglein 2	BF031829
247	MAN1A2	mannosidase, alpha, class 1A, member 2	AL157902
248	CKLFSF6	chemokine-like factor super family 6	NM_017801
249	RNASET2	ribonuclease T2	NM_003730
250	RNASET2	ribonuclease T2	NM_003730
251	DHRS8	dehydrogenase/reductase (SDR family) member 8	NM_016245
252	C11orf15	chromosome 11 open reading frame 15	NM_020644
253	TMEM48	hypothetical protein FLJ10407	NM_018087
254	TPARL	TPA regulated locus	NM_018475
255	AGPAT5	1-acylglycerol-3-phosphate O-acyltransferase 5 (lysophosphatidic acid acyltransferase, epsilon)	NM_018361
256	MFSD1	hypothetical protein FLJ14153	NM_022736
257	PTX1	CDA14	NM_016570
258	NA	Der1-like domain family, member 1	NM_018630
259	ALG5	asparagine-linked glycosylation 5 homolog (yeast, dolichyl-phosphate beta-glucosyltransferase)	NM_013338
260	SCPEP1	serine carboxypeptidase 1	NM_021626
261	SLC38A1	solute carrier family 38, member 1	NM_030674
262	GALNT7	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7 (GalNAc-T7)	NM_017423
263	KIAA1815	KIAA1815	NM_024896
264	RSAFD1	radical S-adenosyl methionine and flavodoxin domains 1	NM_018264
265	TMEM33	transmembrane protein 33	NM_018126
266	LEMD3	LEM domain containing 3	NM_014319
267	FLJ10652	hypothetical protein FLJ10652	NM_018169
268	EIF2AK3	eukaryotic translation initiation factor 2-alpha kinase 3	NM_004836
269	MGC5306	hypothetical protein MGC5306	NM_024116
270	TMEM38B	transmembrane protein 38B	NM_018112

271	SPCS3	signal peptidase complex subunit 3 homolog (S. cerevisiae)	NM_021928
272	SART2	squamous cell carcinoma antigen recognized by T cells 2	NM_013352
273	FLJ13576	hypothetical protein FLJ13576	NM_022484
274	NUP43	nucleoporin 43kDa	NM_024647
275	C1GALT1C1	core 1 UDP-galactose:N-acetylgalactosamine-alpha-R beta 1,3-galactosyltransferase 2	NM_014158
276	TMEM45A	hypothetical protein FLJ10134	NM_018004
277	RAD54B	RAD54 homolog B (S. cerevisiae)	NM_012415
278	ALG6	asparagine-linked glycosylation 6 homolog (yeast, alpha-1,3-glucosyltransferase)	NM_013339
279	C5orf14	chromosome 5 open reading frame 14	NM_024715
280	TMEM49	hypothetical protein DKFZp566I133	NM_030938
281	TXNDC5	thioredoxin domain containing 5	NM_030810
282	NA	TAR DNA binding protein	NM_031214
283	CDA08	T-cell immunomodulatory protein	NM_030790
284	TDE1	tumor differentially expressed 1	AW173623
285	UBA52	ubiquitin C	AF348700
286	MAN1A1	mannosidase, alpha, class 1A, member 1	BG287153
287	NACA	nascent-polypeptide-associated complex alpha polypeptide	AI992187
288	SEC3L1	SEC3-like 1 (S. cerevisiae)	AK023461
289	FLJ22104	hypothetical protein FLJ22104	AK000684
290	LRMP	lymphoid-restricted membrane protein	U10485
291	CAPZB	capping protein (actin filament) muscle Z-line, beta	U03271

Table S2. Summary of RT-PCR results for cell lines 036 and 510

CELL LINE 036						
RQPCR			GENE EXPRESSION			
GENES	Average	STD	CI (95%)	Average	STD	CI (95%)
FLJ10652	1.24	0.17	0.15	1.82	0.22	0.20
SLC38A1	1.27	0.14	0.12	1.82	0.22	0.19
HMMR	0.90	0.14	0.12	1.97	0.34	0.29
ACSL3	1.36	0.25	0.22	1.57	0.15	0.13
POLQ	0.93	0.19	0.17	1.93	0.27	0.24

CELL LINE 510						
RQPCR			GENE EXPRESSION			
GENES	Average	STD	CI (95%)	Average	STD	CI (95%)
CD164	1.60	0.29	0.25	1.86	0.14	0.12
GGH	1.90	0.37	0.32	1.94	0.16	0.14
GLG1	1.46	0.24	0.21	1.88	0.16	0.14
LIPA	1.71	0.32	0.28	2.02	0.12	0.11
MAN1A2	1.39	0.22	0.19	1.86	0.04	0.04
PPT1	1.62	0.31	0.27	1.73	0.11	0.09
SCAMP1	1.59	0.32	0.28	2.03	0.24	0.21
SLC9A6	1.53	0.25	0.22	2.08	0.35	0.31
TRAM1	1.53	0.22	0.19	2.02	0.09	0.08
TM9SF2	1.63	0.25	0.22	2.16	0.23	0.20
SSR1	1.66	0.33	0.29	1.96	0.22	0.19
PAM	2.93	0.18	0.16	1.50	0.08	0.07

Average: Mean value calculated over the 5 low doses; group analyses across doses is justified because these genes did not show a statistically significant slope across the dose range of our study

STD: Standard Deviation

CI: Confidence Interval

Gene specific assays used: GLG1 (Hs00201886_m1), PAM (Hs00168596_m1), PPT1 (Hs00165579_m1), MP21 (Hs00828975_s1), FLJ10652 (Hs00216848_m1), HMMR (Hs00234864_m1), POLQ (Hs00198196_m1), SLC9A6 (Hs00234723_m1). Also see Methods section.

Table S3. Low-dose radiation-responsive genes assigned to sub-cellular locations*

Integral to membrane	TDE2 HMGCR SLC1A1 TMP21 TPARG SLC39A14 TMEM33 ITM2B ALG6 MCP DSG2 AGPAT5 CD53 GLG1 GGH GOLPH2 SLC25A3 SPCS3 PTTG1IP SLC39A8 RNF139 ALCAM LRMP C1ORF8 ATP6V1C2 IFNGR1 SMBP EIF2AK3 SPCS2 ARL6IP5 MAN1A1 ENTPD1 NUP43 ATP2C1 SC5DL FLJ10134 ATP11B CD63 SLC12A2 MS4A1 CD48 TM9SF2 TGFBR3 RSAFD1 FAS TDE1 ITM2A CD38 EBI2 DKFZP564G2022 SSR1 CD83 RANBP2 ABCC4 ACSL1 C11ORF15 CKLFSF6 CDA08 SLC30A1 SLC9A6 SORL1 TAP1 SGCE ATP1B3 RPN2 ATP10D ZMPSTE24 STEAP EDEL2 LRMP LAMP2 ATP6A2 PTPRC PTPRK RNF139 MCP RPN2 LAPTM4A LEMD3 ELOVL5 ZDHHC17 SSR1 CNIH SLC38A1 SLC35A3 TRAM1 TFRC CD48 CD58 CD164 GALNT7 TXNDC4 TLOC1 LYRIC VDP CGI-100 HIP14 MGC8721 SCAMP1 TEB4 TGOLN2 VMP1 COCH SDFR1 CAPZB HLA-B HLA-DRA ITGB1 UCP2 PGRMC1 RANBP1 RDX SLC33A1 SLC39A6 SLC38A
Nuclear Envelope-Endomembrane	SLC9A6 SPCS3 RANBP2 LRMP HMGCR ATP11B SPCS2 STEAP LEMD3 GLG1 TMP21 STCH RPN2 TXNDC4
Endoplasmic Reticulum	SPCS3 SC5DL EDEL2 HMGCR SART2 GRP58 P4HA1 SLC9A6 P44S10 HSPA5 HSP1A1 LRMP TXNDC5 ALG6 TAP1 EIF2AK3 SPCS2 TRA1 ZMPSTE24 MAN1A1 ARL6IP5 RCN1 RTN4 SSR1 RCN2 SLC35A3 TMP21 ALG5 TDE2 SLC1A1 TPARG KTN1 TXNDC4 TLOC1 JWA SEC63 GRP58 SPTLC1 SGSE SLC9A6 TRA1 TAP1 CANX RPN2 CGI-100
Golgi Apparatus	TMP21 ALG5 MAN1A1 ZMPSTE24 SLC35A3 ZDHHC17 GLG1 GMH1 TGOLN2 HSP1A1 AKAP1 AKAP11 COPB GALNT1 SCAMP1 EDEL2 GOLPH2 VDP STEAP
Mitochondrion	DLD MRPS6 UCP2 SLC25A3 NDUFS1 FLJ10618 C14ORF2
Lysosome and Endosome Systems	CD63 HEXB GGH LIPA PPT1 CD164 TM9SF2 TFRC CTSZ LAMP2 CTSS
Cytoplasm	SC5DL HMGCR TMP21 ALG5 AKAP9 CD63 SART2 IFI44 LIPA NDUFS1 G1P2 P4HA1 FUBP1 HSPA5 TXNDC5 TM9SF2 ALG6 UBA52 RTN4 SSR1 GLG1 GOLPH2 SLC25A3 HEXB SPCS3 DLD GGH PAM PTTG1IP HSPA1B PPT1 TMSB10 RARS MRPS6 GRP58 C15ORF24 SLC9A6 LRMP P44S10 EIF2AK3 RPS29 TAP1 TRA1 SPCS2 MAN1A1 HSP1A1 SGCE ZMPSTE24 ARL6IP5 RCN1 STEAP ZDHHC17 RCN2 NACA HSPA1A COCH EPRS FLJ10900 HNRPD ELOV5 LOC5681 P5 PTTG1IP RNASET2 IF144 PCM1 RAD54B UBA52 LOC56851 GIP2 MBNL1 HNRPD COPB TNFRSF6 FUCA1 HEXB PGRMC1 NARS DDX17 RPS28 RPS29 RPL5 RPL17 RPL24 RPL37A ACSL3 ACSL1
Nucleus	FUBP1 BTA1 PNN MBNL1 NUP43 CGI-48 TARDBP GTF3A AR1D5B PHTF1 PTMA HMGA1 PTTG1P MAPK6 MATR3 HNRPD MGC5306 METT23 SFRS7 CREB3L2
Unknown	FLJ20696 UNC50 LRBA DJ97N18.2 FLJ10652 FLJ10900 HTGN29 MGC5306

* Assignments are based on Ingenuity Pathways, GO Ontology and EASE analyses using the candidate 291-gene set; bolded genes are confirmed using the consistent 81-gene set

Table S4. Low-dose radiation-responsive genes assigned to cellular homeostasis functions*

<i>Solute Transport</i>	Cations & Anions	SLC39A14 SLC25A3 SLC39A6 SLC39A8 SLC12A2 SLC30A1 RCN1 RCN2 PAM DLD SLC33A1 SLC16A1 SLC9A6 TXNDC4 GRP58 ATP6VOA2 TFRC TM9SF2 SLC38A1 SLC1A1 FTL FLJ10618 UBA52 METTL3 SWAP70 FLJ22625 DKFZP564K142 SMBP
	Amino Acid Peptide Protein	ARL6IP5 SLC1A1 JWA UBE4A LAPTM4A SEC3L1 ABCC4 VDP SORL1 SCAMP1 TMP21 NUP43 TRAM1
<i>Energy</i>	ATPase Function for Transport	ATP1B3 ATP2C1 ATP11B ATP10D ATP6AP2 ATP6V1C2 STCH P44S10 SMC4L1 DLD TRA1
<i>Metabolism</i>	Fatty acid & Lipid	ACSL1 ACSL3 LIPA ELOV5 SORL1 DLD MINPP1 SQLE P5 ALG5 ALG6 SIAT1 PTPRC HMMR HMGCR SC5DL STCH HIP14 SPTLC1
	Glycoprotein	GALNT1 GALNT7 TXNDC4 RPN1 MAN1A1 RPN2 SGCB SGCE DDOST HEXB ALG5 ALG6
	Carbohydrate	MAN1A1 FUCA1
	Protein Synthesis, Modification, Stability & Degradation	GLG1 GGH PAM HSPA1A RARS RPL17 NARS LOC5681 P5 ZMPSTE24 CAPZB RISC NACA HS2ST1 MRPS6 OAZIN USP1 RPS29 RPS28 RPL5 RPL24 RPL37A RNF13 CTSZ PSMD14 PTPRK TAP1 UBE4A
	RNA & DNA	DDX17 DD5 DHX15 PRPF4B GTF3A SF3B1 PAICS DNAJA1 DNAJB9 RPL5 RNASET2 HNRPD TARDBP SFRS7 ENPP4 EPRS TEB4
<i>Stress Response</i>	Membrane Structure & Cell Signaling	GLG1 RTN4 RDX CD63 HEXB GGH LIPA PPT1 CD164 CD48 HSPA1B FLJ10618 TGFB3 TM9SF2 TFRC TMP21 RANBP2 KPNA2 LAP1B NUP37 JWA MGC14799 CAPZB RPA1 FURIN SORL1 CYP5A1 HSPA5 TGOLN2 TRA1 UBA52 RCN1 HMMR HNF4A GRP58 FLJ10900 PRDX4 VMP1 ZMPSTE24 RANBP2 ROCK1 RCN1 RCN2 SGSE TLOC1 CYP51P2 DHRS8 MATR3 HSPA8 PNN CDA08 NIPA2 SGCB HMGA1 SQLE TRAM1
<i>Cancer Related</i>	Tumori- genesis, & Metastasis	FUBP1 ITGB1 MSH2 LYRIC AKAP9 B2M CANX CD38 CD47 CD48 CD58 CSE1L FUBP1 G1P2 IFNGR1 GRP58 HMGA1 HMMR HSPA5 HSPA8 HSPA1A ITGB1 MCP1 MS4A1 MSH2 PAM PTPRC PTPRK RDX SEC63 TFRC TGFB3 TNFRSF6 TRA1 UCP2 USP1 CTSZ RNF139 DCN SART2 HMGA1 DD5 STEAP

* see Table S3 footnotes

Table S5. Low-dose radiation-responsive genes assigned to signal transduction pathways*

Cell Cycle Control, Survival & Apoptosis	RPL5 PRKCA JWA MATR3 ZRF1 CDKN1A MAPK6 TNFRSF6 PTPRC MGC5306 RNASET2 GRP58 CSE1L DD5 TXNDC4 SMC2L1 PTMA
DNA Repair, Recombination & Replication	BTAF1 MSH2 CREB3L2 CD38 FUBP1 GTF3A SSR1 CSE1L DSG2 EPRS GTF3A ILF2 NACA PCM1 RARS RCN2 HSPCA1A HSPA5 RAD54B RANBP2 SF3B1 POLQ FUBP1 H2AFX MSH2 RAD54B
Immune Response & Cell Signaling	TGFBR3 ILF2 HLA-DRA GRP58, HLA-B HLA-DRA CD44 SDFR1 PRDX4 ITGB1 IFNGR1 ITGA4 LAMP2 ROCK1 TDE1 TNFRSF6 GIP2 GGH GLG1 CD48 CD58 CD83 HMGCR PAM TFRC PTPRC ALCAM CAPZB OAZIN IFI44 SDFR1 CD164 MCP1 HMMR MS4A1 SART2 CD38 CD53 RNF13 RNF139 MAPK6 CNIH CTSS PRBP4 AKAP1 AKAP 9 AKAP11 TAP1 EB12 ENTPD1 GIP2 KTN1
Unknown	FLJ20696 UNC50 RPN4 FLJ10525 C6ORF6 TPARL KIAA0186 KIAA0033 KIAA0102 KIAA0922 KIAA1815 AF31104 C1ORF8 C11ORF15 CGI-100 CKLFSF6 COCH DJ97N18.2 FLJ10652 FLJ10900 GMH1 HTGN29B LOC56851

* see Table S3 footnotes