# Dose-dependent expression changes of early response genes to ionizing radiation in human lymphoblastoid cells

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**Abstract.** The sensitivity of cancer cells as well as normal cells in response to ionizing radiation (IR) is believed to be associated with the early inducible expression of specific genes. Using cDNA microarray technology, here we explored and compared the global transcriptional changes in human lymphoblastoid AHH-1 cells irradiated with 0.05-, 0.2-, 0.5-, 2.0- and 10-Gy doses of γ-rays 4 h after exposure. A dose as low as 0.05 Gy was efficient in inducing a transcriptional response including the up-regulation of 25 genes, some of which are involved in signal transduction pathways, e.g. *BMPR2*, *GPR124*, *MAPK8IP2* and *AGGF1*, and the down-regulation of 18 genes. Expression of some genes was altered only at a specific dose. Most importantly, we discovered a number of radiation-response genes, e.g. DNA repair gene *XPC*, tumor protein p53 inducible protein 3 gene (*TP53I3*), immediate early response 5 gene, whose transcriptional levels were increased or depressed by IR in a dose-dependent trend within the dose range 0.05-10 Gy. The dose-dependent induced expression of *TP53I3* and *XPC* was confirmed by Northern blot analyses. Using quantitative real-time PCR, we further confirmed that *XPC* gene induction was dose dependent as well as time dependent, reaching a peak 4 h post-2 Gy and 10 h post-0.05 Gy. The maximum induced expression level of the *XPC* gene was higher after 2 Gy (3.2-fold) than 0.05 Gy (1.93-fold). The identification of these radiation-inducible genes, especially those exhibiting a dose-dependent response, not only expands our knowledge of the mechanisms underlying the diverse biological effects induced by IR, but provides candidates for developing novel biomarkers of radiation injury.

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*Key words:* radiation effect, gene expression, radiation-inducible gene, biomarker

## Introduction

It is well documented that the biological effects induced by exposing mammalian cells to ionizing radiation (IR) are closely associated with radiation doses and dose rates. Larger doses which are used for radiotherapy of cancers lead to more severe injury, while low-dose radiation (i.e. <0.5 Gy) may result in a complex scenario of cellular responses that are either protective or supra-lethal according to the dose, e.g. adaptive response (1-7), hyper-radiosensitivity phenomenon as well as the inverse dose-rate effect (8-10). These low-dose radiation responses cannot thus be entirely predicted by extrapolation from the data obtained from large acute exposures. It has been suggested that some specific mechanistic pathways are induced by exposure to a specific dose range of IR.

The inducible alteration in gene expression is a fundamental molecular event of mammalian cells in response to ionizing radiation, and the fate of cells will at least partially depend upon the inducible changes in expression of some genes involved in these complex regulatory pathways resulting in cell cycle delays, cell killing or apoptosis and DNA repair (11-15). The cDNA microarray technology allowing a large-scale expression profiling analysis can provide tremendous information for elucidating the complex cellular response to radiation. Until now, a great number of radiation-inducible target genes have already been identified. Some of the inducible genes were identified in cells exposed to high and even supra-lethal doses of IR (11,16-23), low doses or low dose rates (13,23-28). These reports provide very valuable information for understanding the precious mechanisms of the diverse effects of IR. A simultaneously comparative analysis of gene responses to radiation from low, medium to high doses should be more informative for identifying the inducible genes with a dose-dependent expression. The aim of the present study was to identify such genes responding to low-dose radiation or with an inducible expression change in a dose-dependent manner towards the elucidation of the mechanisms of radiation effects and the development of novel biomarkers for the identification or classification of individuals with different radiosensitivity or for predicting normal tissue injury of patients during radiotherapy.

For this purpose, we used cDNA microarray technology to compare the global transcriptional changes of human

lymphoblastoid AHH-1 cells 4 h after exposure to γ-rays of graded doses from 0.05-10 Gy. Our study identified a number of genes with dose-dependent expression trends and also a set of low-dose responsive genes. Using Northern blot hybridization and sensitive quantitative real-time PCR, further analyses of changes in expression of the *TP53I3* gene and DNA repair gene *XPC* also revealed dose- and/or time-dependent responses.

## Materials and methods

*Cell culture and irradiation*. The human lymphoblastoid AHH-1 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/ml of penicillin and 100 μg/ml of streptomycin at 37˚C in a humidified, 5% $CO_2$ atmosphere. Exponentially growing cells were irradiated at room temperature using a cobalt-60 γ-ray source at a dose rate of either 1.7 Gy/min (for doses ≥0.5 Gy) or 0.96 cGy/min (for doses 0.2 and 0.05 Gy). For sham-radiation control, the cell cultures were set in the radiation room for an identical time as a corresponding dose while the cobalt source remained under water and sealed (without exposure). After irradiation, cells were cultured for 4 h and then harvested to prepare RNA extracts.

*RNA isolation and microarray analysis*. Total RNA was isolated from irradiated and sham-irradiated cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Twenty μg of total RNA from irradiated or sham-irradiated cells was used as a template for generating Cy3- or Cy5-labeled cDNA probes after *in vitro* reverse transcription reaction (RT). Experimental conditions for the RT reaction and cDNA hybridization were performed as previously described (29). BiostarH-140s cDNA microarray (Biostar, Shanghai, P.R. China) was used which contains cDNA probes corresponding to 14,100 specific human genes that were scanned by ScanArray 4000 (Packard/Perkin Elmer, MN, USA). The intensities of the fluorescent images were captured, and the data were processed using GenePix Pro 3.0 imaging software (Axon Instruments, Foster City, CA, USA). Expression ratios were normalized to those of a set of forty housekeeping genes with a theoretical ratio of 1.0. For each dose, two independent microarray hybridization experiments were carried out using a mix of Cy3- and Cy5-labeled cDNA from control and irradiated AHH-1 cells, respectively. Fluoro-chromes were swapped between the first and second experiment. Data for a spot were defined as effective if the raw intensity value of both Cy3 and Cy5 was >200 after back-ground correction.

*Semi-quantitative RT-PCR and quantitative real-time PCR*. RT-PCR was used to semi-quantify the mRNA expression level of genes. Briefly, first-strand cDNA was reversely transcribed from 2 μg of total RNA using an oligo-$(dT)_{15}$ primer and MMLV reverse transcriptase (Clontech) in a 20-μl reaction mixture. The primer pairs and PCR conditions for each gene are listed in Table I. The number of PCR cycles used allowed the quantification without reaching the amplifying plateau for PCR products. As internal standard, a fragment of human endogenous ß-actin was amplified simultaneously in each PCR reaction. PCR products were resolved on a 2.0% agarose gel, and the bands were visualized by ethidium bromide staining. The cDNA bands were semi-quantified by densitometry measurement. The ratio of the

Table I. Primer pairs and PCR conditions for each gene amplification.

| Gene | Primer pairs | PCR conditions | Product (bp) |
|---|---|---|---|
| BMPR2 | F: 5' TTTCTGCCCCATCTCCCATACT 3'<br>R: 5' CTCCCCAAAACCAGAAGCAATA 3' | 94˚C for 30 sec, 54˚C for 30 sec,<br>72˚C for 20 sec; 30 cycles | 247 |
| Cx43 | F: 5' GCTGAGCCCTGCCAAAGACTGT 3'<br>R: 5' TCGGGGAAATCAAAAGGCTGTG 3' | 94˚C for 30 sec, 57.5˚C for 30 sec,<br>72˚C for 20 sec; 30 cycles | 255 |
| NOL6 | F: 5' TGCCCCCAGCCCAGGTTAC 3'<br>R: 5' GGCACGGGCGGAAGAAGTC 3' | 94˚C for 30 sec, 59.1˚C for 30 sec,<br>72˚C for 20 sec; 28 cycles | 333 |
| LYK5 | F: 5' AGGCGGTGGGTCTCGGAAAAGT 3'<br>R: 5' AGCTCGCCCTGCAAGAATGTTA 3' | 94˚C for 30 sec, 59˚C for 30 sec,<br>72˚C for 20 sec; 30 cycles | 294 |
| DKEZP686P0196 | F: 5' GGCATCCAGGCGTTTCCATACA 3'<br>R: 5' CACTGCAGCCCCTCTCATCACA 3' | 94˚C for 30 sec, 57˚C for 30 sec,<br>72˚C for 20 sec; 32 cycles | 295 |
| MAPK8IP2 | F: 5' CTCCAGCACCGAGTCCTTTGG 3'<br>R: 5' GCATGTTGAAGCCACGGAACCA 3' | 94˚C for 30 sec, 60˚C for 30 sec,<br>72˚C for 20 sec; 32 cycles | 182 |
| XPC | F: 5' AGG CAA AAC ACA TGG ACC AG 3'<br>R: 5' AGT AGA CCG CTT CTC CAC GAC 3' | 94˚C for 30 sec, 56.5˚C for 30 sec,<br>72˚C for 20 sec; 25 cycles | 335 |
| ß-actin | F: 5' GCCAGGTCCAGACGCAGGAT 3'<br>R: 5' CGGTCCAGGTGTGTCCTA 3' | 94˚C for 30 sec, 56.2˚C for 30 sec,<br>72˚C for 20 sec; 22 cycles | 127 |

F, forward; R, reverse

specific gene intensity signal to the ß-actin intensity signal was used for semi-quantitative analysis expressed by the relative mRNA level.

To quantify the mRNA level of the *XPC* gene, quantitative real-time PCR was performed on CFD-3240 Chromo4™ continuous fluorescence detector system (MJ Research, Inc., Watertown, MA, USA), using 10 $\mu$l of DyNAmo™ SYBR®-Green qPCR master mix containing the modified *Thermus brockianus* hot start DNA polymerase (Finnzymes Oy, Espoo, Finland), 0.2 $\mu$M of each primer and 2 $\mu$l cDNA template (obtained after RT as described above) in a 20-$\mu$l final volume. The amplification was started by a 10-min incubation at 95˚C to denature the template cDNA and activate Taq polymerase, then followed by 34 cycles consisting of denaturation at 95˚C for 20 sec, annealing at 58.8˚C for 20 sec, and synthesis at 72˚C for 20 sec. To measure the PCR products generated in the presence of SYBR-Green I, a $T_m$ analysis was performed by increasing the temperature from 65-90˚C at a linear transition rate of 0.2˚C/sec. Fluorescence was monitored continuously while the temperature was increased and data were collected using Option Monitor2 software (MJ Research, Inc.). The standard curve was plotted using Origin 5.0 software (Microcal Software, Inc., Northampton, MA, USA). The cycle number at which the fluorescent signal crossed the detection threshold was denoted as the threshold cycle (CT). CT values obtained for the *XPC* gene were normalized using the ß-actin gene as the internal standard. Each PCR reaction was run in triplicate in three independent experiments.

Table II. The numbers of genes with changed transcription levels in the irradiated AHH-1 cells.

| Expression changes | 0.05 Gy | 0.2 Gy | 0.5 Gy | 2.0 Gy | 10.0 Gy |
|---|---|---|---|---|---|
| Up-regulated | 25 | 21 | 30 | 32 | 26 |
| Down-regulated | 18 | 62 | 45 | 46 | 219 |

*Northern blot analysis*. Total RNA (15 $\mu$g) was fractionated on a 1.2% formaldehyde agarose gel, transferred onto a Duralon-UV membrane (Stratagene), and hybridized to [$\alpha$-$^{32}$P]dCTP-labeled *Cx* 43, *P53IP3* or *XPC* cDNA probe. The glyceraldehyde 3'-phosphate dehydrogenase (*GAPDH*) cDNA probe was used as the internal control for the quantity of the loaded RNA sample.

*Western blot analysis*. The cells were harvested and washed twice in ice-cold phosphate-buffered saline. Cell pellets (0.5-1x10$^6$) were incubated in 100 $\mu$l of Master lysis buffer (M-PER™ mammalian protein extraction reagent) (Pierce, Rockford, IL, USA) for 5-8 min at room temperature. After centrifugation for 15 min at 4˚C, whole protein extract was harvested and the protein concentration was determined. A total of 20 $\mu$g of proteins was resolved on 8% SDS/PAGE and transferred onto a polyvinylidene fluoride (PVDF) membrane. The immunohybridization was performed using the anti-Connexin 43 polyclonal antibody (#66-0700, Zymed), or

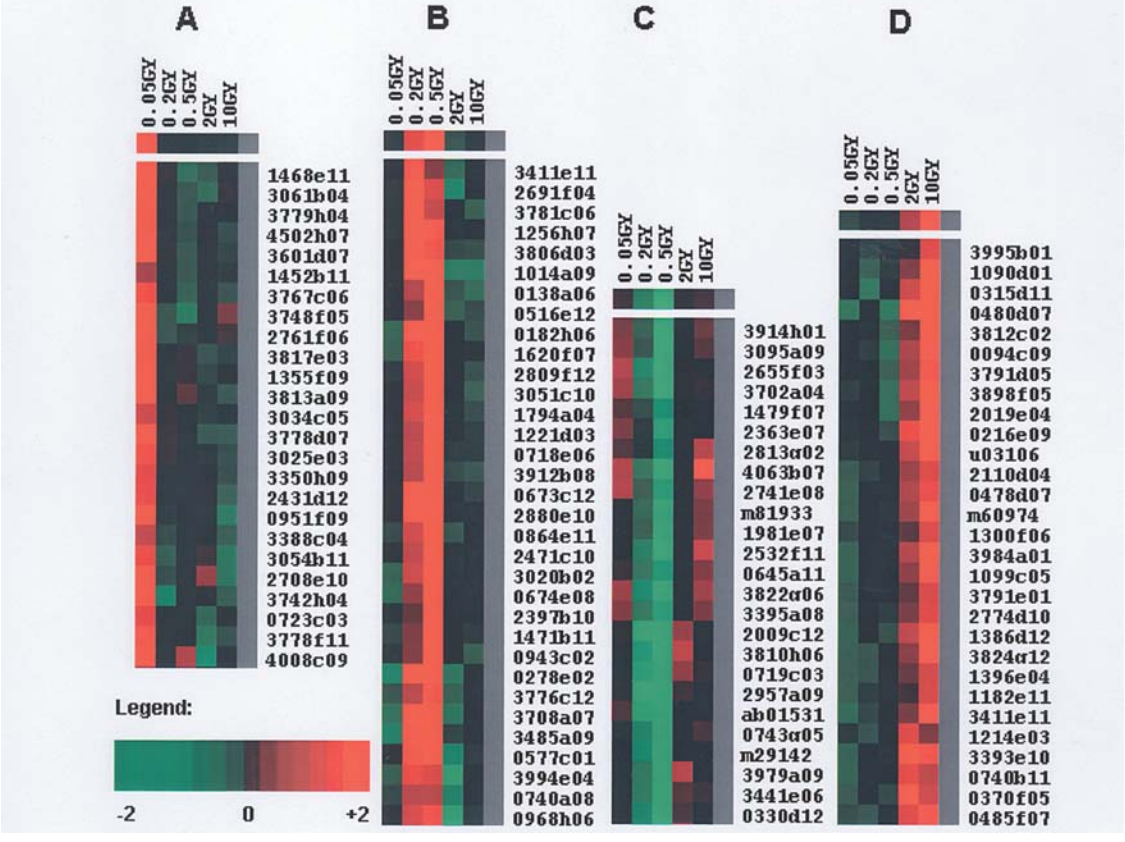


Figure 1. The cluster analysis representative of transcriptional profiles of AHH-1 cells 4 h after exposure to 0.05-, 0.2-, 0.5-, 2- and 10-Gy γ-rays. The cluster analysis (K-mean) was performed using Genomic Profiler software. This figure shows the genes whose expression was increased (red color; A, B, D) or decreased (green color, C) by radiation as compared with those of the sham-irradiated cells. Overlapping of the increased expression genes was also observed between 0.2 and 0.5 Gy (B) or 2 and 10 Gy (D), as well as that of the decreased expression genes between 0.2 and 0.5 Gy (C).

Table III. The list of up-regulated genes by 0.05-Gy low-dose irradiation.

| Genbank no. | Gene definition | Changed fold | | |
|---|---|---|---|---|
| | | Exp 1 | Exp 2 | Average |
| NM_000828 | Glutamate receptor, ionotrophic, AMPA 3 (GRIA3) | 1.960 | 2.048 | 2.004 |
| NM_022917 | Nucleolar protein family 6 (RNA-associated) (NOL6) | 2.042 | 2.034 | 2.038 |
| NM_020193 | Chromosome 11 open reading frame 30, ENT domain containing | 2.061 | 2.101 | 2.081 |
| NM_001204 | Bone morphogenetic protein receptor, type II (serine/threonine kinase) (BMPR2) | 2.226 | 2.084 | 2.155 |
| NM_000165 | Gap junction protein, α 1, 43-kDa (connexin 43) (GJA1) | 2.146 | 2.160 | 2.150 |
| NM_001003954 | Annexin A13 (ANXA13) | 2.070 | 2.252 | 2.161 |
| BX436525 | Unknown | 2.382 | 2.561 | 2.422 |
| BX537946 | EPH_lbd, FN3, TyrKc and SAM domain-containing | 1.924 | 3.588 | 2.506 |
| NM_016524 | Homo sapiens B/K protein (LOC51760) | 2.201 | 2.806 | 2.506 |
| BC043641 | Homo sapiens protein kinase LYK5 | 2.519 | 2.515 | 2.517 |
| NM_032777 | G protein-coupled receptor 124 (GPR124) | 2.524 | 2.516 | 2.520 |
| NM_012324 | Mitogen-activated protein kinase 8 interacting protein 2 (MAPK8IP2), or the JNK-interacting protein 2 (JIP2) | 2.508 | 2.546 | 2.527 |
| BC028683 | Human PRP39 pre-mRNA processing factor 39 homolog (yeast) | 2.340 | 4.064 | 3.202 |
| BX538266 | Unknown, cDNA DKFZp686P0190 | 3.240 | 3.212 | 3.226 |
| NM_018046 | Angiogenic factor with G patch and FHA domains 1 (AGGF1) | 3.846 | 2.812 | 3.330 |

anti-ß-actin polyclonal antibody (#I-19, Santa Cruz) and then the IgG-HRP secondary antibody (Zhongshan Company, Beijing, P.R.China). Bands were visualized using chemiluminescence (Santa Cruz) according to the manufacturer's instructions.

## Results

*Comparison of transcriptional profiles of AHH-1 cells after exposure to different doses of γ-rays*. Two independent BiostarH-140s microarray hybridizations were carried out for each radiation dose with Cy3- or Cy5-labeled cDNAs obtained after RT of RNA extracted from human lymphoblastoid AHH-1 cells harvested 4 h after 0.05, 0.2, 0.5, 2.0 and 10.0 Gy of γ-ray exposure or sham-irradiation. The genes modulated at least 2-fold in average but at least 1.9-fold in each individual microarray experiment were considered as reliable radiation-responsive genes. By choosing these criteria of analysis, the number of radiation-inducible genes ranged from 43-245 genes, i.e. ~0.5-2% of the total genes on the microarray, according to the dose tested (Table II). Up- and down-regulated genes were almost equally distributed in the 0.05-, 0.5- and 2-Gy sets. In contrast, the transcriptional alterations after 10 Gy, which is a supra-lethal dose, included mostly down-regulations (219/245, 90%). Fig. 1 shows the clustering analysis representative of the genes with altered expression. It is clear that a certain set of genes was induced by a specific dose or dose range.

Even a dose as low as 0.05 Gy was effective in eliciting a transcriptional response (Table III). Some of these genes with a 2-fold increased expression induced by 0.05 Gy are involved in signaling pathways (BMPR2, GPR124, JIP2/MAPK8IP2 and AGGF1), intercellular transport/cell-cell communication (ANXA13, Connexin 43) or ion channeling/neurotransmitting (GRIA3/GLUR3).

A set of 0.2-Gy-inducible genes was also identified. The 0.2-Gy up-regulated genes include those belonging to i) signal transduction: RAS protein activator-like 2 (RASAL2), human lymph node homing receptor mRNA, killer cell lectin-like receptor subfamily C member 2 (KLRC2), vitronectin (serum spreading factor somatomedin B complement S-protein) (VTN) and plexin A4 (PLXNA4); ii) transcription or DNA-binding factors: retinoblastoma-associated factor 600, ring finger protein 2 (RNF2); iii) cytoskeleton and cell movement: prickle-like 2 (*Drosophila*) (PRICKLE2), Homo sapiens a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif 1 (ADAMTS1), radial spokehead-like 3 (RSHL3); and iv) cell adhesion: latent transforming growth factor ß binding protein 2 (LTBP2), protocadherin α 9 (PCDHA9) and protocadherin 18 (PCDH18).

We observed few genes showing a significant alteration of expression simultaneously at both 0.2- and 0.5-Gy doses at the 2-fold threshold. However, if the threshold was reduced to 1.8-fold, some genes were affected by both 0.2 Gy and 0.5 Gy. These included the up-regulated genes such as

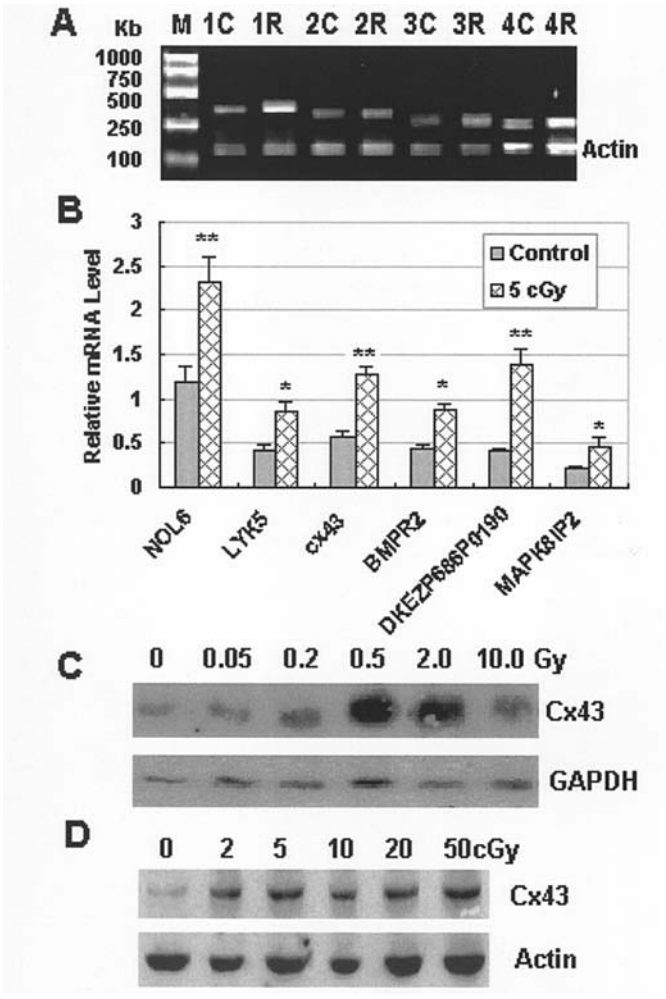


Figure 2. Confirmation of gene expression changes selected from the microarray analysis by semi-quantitative RT-PCR. (A) Representative electrophoresis gel image of RT-PCR products amplified from the sham-control (C) and 0.05-Gy-irradiated (R) samples prepared at 4 h after irradiation. M, DNA marker; 1, NOL 6; 2, LYK5; 3, connexin 43 (Cx 43), 4, BMPR2. (B) Semi-quantification of PCR products. The human ß-actin gene was used as the internal standard. The relative mRNA level was calculated by the ratio of the selected gene amplification product to the ß-actin gene amplification product. Each bar represents the means ± SD from 3 independent experiments. Student's t-test: $^{*}P<0.05$, $^{**}P<0.01$ as compared with the sham-irradiated samples. (C) Northern blot analysis of Cx 43 mRNA expression 4 h after exposure to different doses of γ-rays. (D) Western blot analysis of Cx 43 protein expression 4 h after exposure to different doses of γ-rays.

tumor protein p53 inducible protein 3 (*TP53I3*), xeroderma pigmentosum complementation group C (*XPC*), the human chromodomain helicase DNA binding protein 6 gene (*CHD6*), mitogen-activated protein kinase kinase kinase 7 interacting protein 2 gene (*MAP3K7IP2*), G protein-coupled receptor 56 gene (*GPR56*), Homo sapiens protocadherin α 9 (*PCDHA9*) and several unknown genes; and the down-regulated genes such as vav 1 oncogene (*VAV1*), phospho-mevalonate kinase (*PMVK*), lymphocyte cytosolic protein 1 (L-plastin) (*LCP1*), fibrinogen-like 2, human egl nine homolog 2 (*EGLN2*), protein tyrosine phos-phatase type IVA, member 2 (*PTP4A2*) and serine (or cysteine) proteinase inhibitor, clade D (heparin cofactor) member 1, and some unknown genes.

There were 245 genes showing an altered expression induced by 10 Gy, the largest number of radiation-inducible genes among all the investigated doses. Most of these 10-Gy-responsive genes (90%) were depressed. The detailed lists of the altered expression genes at 2 Gy and 10 Gy are available upon request.

Most importantly, we identified a number of genes that exhibited a dose-dependent trend of increased (Table IV), or depressed (Table V) expression induced by IR, i.e. the extent of up- and down-regulation augmented along with the increase of radiation dose.

*Validation of altered expression of radiation-inducible genes selected in microarray assay*. The altered expression of 0.05-Gy low-dose-responsive genes *BMPR2*, Connexin 43, *LYK5*, *NOL6*, *MAPK8IP2* and cDNA clone DKEZP686P0190 were validated by semi-quantitative RT-PCR (Fig. 2A and B). Our microarray analysis demonstrated that the Connexin 43 gene was up-regulated by the 0.05-Gy low dose of γ rays as previously reported after exposure to low-dose α particles (25). We further confirmed this induction of Connexin 43 at the transcriptional level by Northern blot hybridization (Fig. 2C) as well as at the translational level by Western blot analysis (Fig. 2D).

Xeroderma pigmentosum complementation group C gene (*XPC*) and tumor protein p53 inducible protein 3 gene (*TP53I3*) were shown up-regulated in a dose-dependent trend within the dose range 0.05-10 Gy in our microarray assays. The Northern blot analyses confirmed a clear dose-dependent inducible expression of both *XPC* (Fig. 3A) and *TP53I3* (Fig. 3B). Using quantitative real-time PCR, we further quantified the expression of the *XPC* gene after radiation. As shown in Fig. 3C, the expression level of *XPC* was increased in a clear dose-dependent manner 4 h after exposure with different doses from 0.05-10 Gy. *XPC* gene induction was also shown to be time-dependent (Fig. 3D), reaching a peak 4 h after 2 Gy, and then declining at 10 h. The 0.05-Gy low dose was also effective in inducing *XPC* expression, with a maximum increase at 10 h after IR (1.93-fold), which occurred at later times and was lower than after 2-Gy irradiation (3.2-fold) (Fig. 3D).

## Discussion

The severities of IR-induced biological effects in mammalian cells are highly associated with the radiation quality (LET, nature of the induced lesion) as well as radiation doses or dose rates. Even other factors such as cell cycle phase, radical scavenger level, DNA repair status influence the fate of irradiated cells. Tissue injury or a cellular reaction may be induced by doses over a threshold, with the most severe cytotoxic effects or tissue reaction caused by acute exposure to high doses. With regard to low-dose exposure, a more complex scenario of cellular responses has been portrayed as suggested by the existence of the adaptive response (1-7), bystander effects (3,4,11,30-32) and hyper-radiosensitivity phenomenon (8,9,33,34). It is believed that these diverse cellular responses to IR are at least partially dependent on the specific induction of some genes. Here we used microarray to simultaneously investigate the global transcriptional

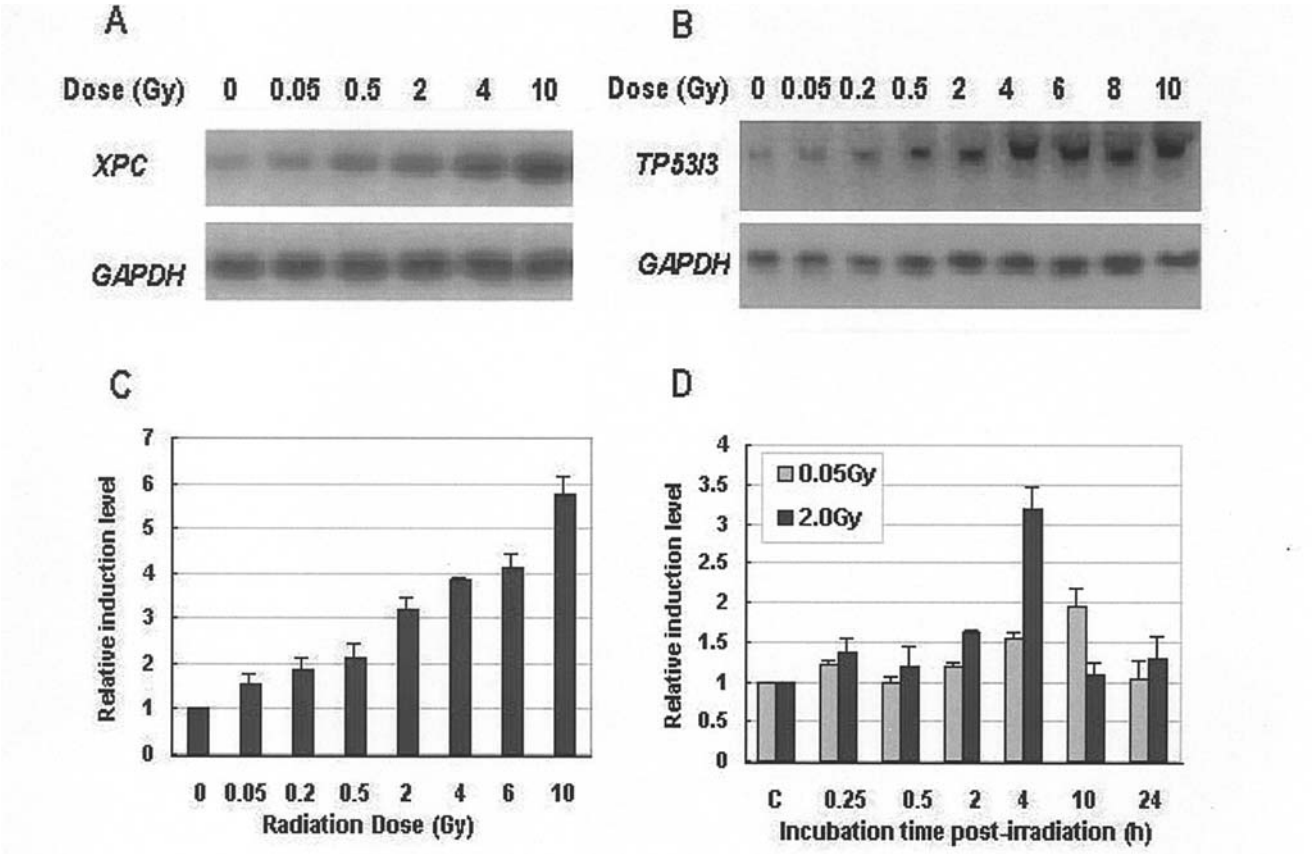


Figure 3. Validation of radiation-induced expression changes of the *TP53I3* and *XPC* genes in AHH-1 cells. (A) Dose-responsive change of *TP53I3* gene expression detected by Northern blot hybridization. (B) Dose-responsive change of *XPC* gene expression detected by Northern blot hybridization. (C) Dose-responsive change of *XPC* gene expression detected by quantitative RT-PCR. (D) Time-dependent change of *XPC* gene expression in AHH-1 cells detected by quantitative RT-PCR. Each bar of data represents the means ± SD from 3 independent experiments.

Table IV. The list of up-regulated genes with a dose-dependent tendency.[a]

| Accession no. | Definition | 0.05 Gy | 0.2 Gy | 0.5 Gy | 2.0 Gy | 10 Gy |
|---|---|---|---|---|---|---|
| AK091115 | Highly similar to mRNA for seven transmembrane protein TM7SF3 | 0.875 | 1.458 | 2.044 | 2.339 | 1.946 |
| NM_002527 | Neurotrophin 3 (NTF3) | 0.989 | 1.452 | 1.801 | 1.965 | 2.395 |
| BE889962 | Unknown | 1.149 | 1.683 | 2.149 | 2.412 | 2.677 |
| NM_004879 | Etoposide-induced 2.4 mRNA (EI24) | 1.022 | 1.543 | 1.652 | 2.624 | 2.727 |
| NM_023039 | Ankyrin repeat, family A (RFXANK- like), 2 (ANKRA2) | 1.071 | 1.377 | 1.623 | 2.556 | 2.835 |
| NM_147188 | F-box protein 22 (FBXO22) | 1.248 | 1.454 | 1.686 | 2.613 | 2.846 |
| NM_016545 | Immediate early response 5 (IER5) | 1.071 | 1.354 | 1.608 | 2.018 | 2.876 |
| NM_001001342 | Biogenesis of lysosome-related organelles complex-1, subunit 2 (BLOC1S2) | 1.241 | 1.560 | 1.609 | 2.176 | 3.321 |
| D87328 | mRNA for HCS | 1.343 | 1.571 | 1.633 | 2.223 | 3.464 |
| BE896331 | Unknown | 1.075 | 1.241 | 1.542 | 2.309 | 3.563 |
| AK055972 | cDNA FLJ31410 fis, highly similar to MDS025 mRNA | 0.896 | 1.243 | 1.442 | 2.173 | 3.622 |
| NM_004628 | Xeroderma pigmentosum complementation group C (XPC) | 1.218 | 1.830 | 1.923 | 2.903 | 3.752 |
| BU729993 | UI-E-CK1-afi-b-20-0-UI | 0.919 | 1.781 | 2.382 | 2.727 | 3.882 |
| AK123575 | Unknown | 1.037 | 1.476 | 2.494 | 2.341 | 4.056 |
| NM_004881 | Tumor protein p53 inducible protein 3 (TP53I3) | 0.992 | 1.878 | 1.984 | 6.872 | 5.461 |

[a]The data are the average from two independent microarray experiments.

Table V. The list of down-regulated genes with dose-dependent tendency.[a]

| Genbank no. | Definition | 0.05 Gy | 0.2 Gy | 0.5 Gy | 2.0 Gy | 10 Gy |
|---|---|---|---|---|---|---|
| NM_012218 | Interleukin enhancer binding factor 3 (ILF3) | 0.838 | 0.590 | 0.315 | 0.330 | 0.151 |
| AB058767 | Unknown | 0.740 | 0.632 | 0.293 | 0.330 | 0.156 |
| XM_035299 | Zinc finger, SWIM domain-containing 6 | 0.783 | 0.571 | 0.405 | 0.534 | 0.173 |
| NM_002444 | Moesin (MSN), mRNA | 0.680 | 0.537 | 0.319 | 0.386 | 0.189 |
| AK090828 | Unknown | 0.879 | 0.840 | 0.353 | 0.621 | 0.197 |
| NM_002298 | Lymphocyte cytosolic protein 1 (LCP1) | 0.930 | 0.512 | 0.297 | 0.372 | 0.202 |
| AK092904 | Unknown | 0.846 | 0.626 | 0.511 | 0.443 | 0.221 |
| AK127479 | Unknown | 0.744 | 0.703 | 0.332 | 0.345 | 0.226 |
| NM_018639 | WD repeat and SOCS box-containing 2 (WSB2) | 0.929 | 0.620 | 0.676 | 0.483 | 0.240 |
| BX647112 | Unknown | 0.898 | 0.618 | 0.535 | 0.488 | 0.251 |
| NM_018031 | WD repeat domain 6 (WDR6) | 0.768 | 0.780 | 0.530 | 0.622 | 0.261 |
| NM_025182 | Unknown | 0.919 | 0.662 | 0.594 | 0.571 | 0.262 |
| AK092677 | Unknown | 1.047 | 0.706 | 0.569 | 0.377 | 0.283 |
| NM_016343 | Centromere protein F (mitosin) (CENPF) | 0.784 | 0.859 | 0.739 | 0.522 | 0.290 |
| AK022952 | Unknown | 1.173 | 0.860 | 0.722 | 0.616 | 0.308 |
| NM_005347 | Heat shock 70-kDa protein 5 (HSPA5) | 1.149 | 0.753 | 0.639 | 0.547 | 0.321 |
| NM_001976 | Enolase 3 (ß, muscle) (ENO3) | 0.983 | 0.588 | 0.626 | 0.438 | 0.336 |
| BC065016 | Human AFG3 ATPase family gene 3-like 2 | 1.119 | 0.864 | 0.686 | 0.557 | 0.338 |
| AK025276 | Unknown | 1.157 | 0.625 | 0.550 | 0.519 | 0.339 |
| AK000498 | Unknown | 0.831 | 0.630 | 0.761 | 0.551 | 0.355 |
| NM_152840 | Hermansky-Pudlak syndrome 4 (HPS4) | 1.205 | 0.777 | 0.516 | 0.338 | 0.366 |
| AK091498 | Unknown | 1.318 | 0.647 | 0.684 | 0.577 | 0.372 |
| BX538251 | Unknown | 0.915 | 0.899 | 0.403 | 0.631 | 0.373 |
| BC020516 | Interferon regulatory factor 2 binding protein 2 | 0.941 | 0.821 | 0.884 | 0.554 | 0.388 |
| NM_015981 | Calcium/calmodulin-dependent protein kinase II α (CAMK2A) | 0.761 | 0.656 | 0.581 | 0.596 | 0.390 |
| BC012731 | Human Mak3 homolog (S. cerevisiae) | 0.885 | 0.616 | 0.460 | 0.524 | 0.391 |
| BG115862 | Unknown | 0.846 | 0.776 | 0.803 | 0.645 | 0.394 |
| AK128054 | Unknown | 0.941 | 0.827 | 0.538 | 0.683 | 0.413 |
| BM664759 | Unknown | 0.877 | 0.766 | 0.770 | 0.691 | 0.422 |
| NM_015059 | Homo sapiens talin 2 (TLN2), mRNA | 0.882 | 0.722 | 0.717 | 0.539 | 0.450 |

[a]The data are the average from two independent microarray experiments.

changes occurring during the early response to irradiation (4 h) in human lymphoblastoid AHH-1 cells exposed to different doses from 0.05-10 Gy. Our data demonstrated that the alterations in gene expression induced by IR were actually related with the radiation dose.

It has been widely reported that an adaptive response can be induced by a few cGy in various cell lines including AHH-1 cells, while its relevant molecular mechanism is still unclear. Our microarray data showed that 0.05 Gy was effective in inducing the expression of a set of genes (Table III), including those involved in signal transduction pathways regulating the proliferation in various cell types, among which MAPK8IP2 was originally described as a member of the putative scaffold protein family implicated in the regulation of JNK signaling modules and named JIP2 [the c-Jun $NH_2$-terminal kinase (JNK)-interacting protein 2] (35). MAPK8IP2 is a scaffold for the p38 mitogen-activated protein (MAP) kinase cascade in mammalian cells because of its binding to the Rac target Mixed lineage kinase 3 (MLK3), the MAP kinase kinase MKK3, and the p38 MAP kinase (36,37). Furthermore, it can bind to and be modified by the Rac GEFs Tiam1 and Ras-GRF1, which are guanine nucleotide exchange factors (GEFs) that activate the Rac GTPase, suggesting a mechanism connecting a scaffold for the p38 kinase cascade to upstream regulatory proteins (36,37). It is well known that p38 MAP kinases, including p38α, p38ß, p38γ, and p38δ, play an important role in mediating cellular responses to both environmental stresses and proinflammatory cytokines. The p38 MAPK signaling pathway was suggested to play a role in the adaptive response of low-dose radiation via a rapid and robust circuitous feedback signal transduction pathway involving

activation of protein kinase C (PKC)α and p38MAPK in mouse m5S cells (38). The MAPK pathway was also shown recently to be involved in bystander response (39). Our observation of the inducible expression of MAPK8IP2/JIP2 by 0.05 Gy in human AHH-1 cells further implies the involvement of the p38 MAPK signaling pathway in mediating cellular responses to low-dose radiation.

Connexin 43, one of the 0.05-Gy-inducible genes, has been described to be up-regulated by α-particles at a dose as low as 0.03 Gy in normal human skin fibroblasts (25). In the present study, Connexin 43 and another intercellular transport/communication component ANXA13 were also found to be up-regulated by 0.05 Gy in AHH-1 cells, further suggesting the involvement of intercellular communication in the low-dose response.

We recently reported a set of 0.5-Gy-responsive genes in AHH-1 cells (29). A radiation dose of ~0.5 Gy was shown to result in a hyper-radiosensitivity response (HRS), another low-dose radiation-induced biological effect defining the phenomenon by which mammalian cells respond to radiation doses <0.5 Gy as compared to a high-dose response. Here we further revealed a number of 0.2-Gy-responsive genes, including those involved in signal transduction pathways or receptor networks, cytoskeleton and cell movement, cell adhesion and transcription or DNA-binding factors. If the 2-fold threshold of increase or decrease in signal intensity was adjusted to 1.8-fold, a series of genes could be considered to be modulated by both 0.2- and 0.5-Gy irradiation, including up-regulated genes (*TP53I3*, *XPC*, *CHD6*, *MAP3K7IP2*, *GPR56*, *PCDHA9*) and down-regulated genes including DNA repair gene *XRCC4*, vav 1 proto-oncogene (*VAV1*), phosphomevalonate kinase gene (*PMVK*), lymphocyte cytosolic protein 1 (L-plastin) gene (LCP1), fibrinogen-like 2, human egl nine homolog 2, protein tyrosine phosphatase type IVA member 2 gene (*PTP4A2*) and serine (or cysteine) proteinase inhibitor gene, clade D (heparin cofactor) member 1 gene. From these, *CHD6* was further confirmed to have induced expression by 0.2 or 0.5 Gy in A549 and HeLa cells (29), while the A549 cells were documented to exhibit hyper-radiosensitivity (HRS) (34). SiRNA-mediated depression of CHD6 resulted in an increased radioresistance to radiation up to 2 Gy, but barely affected the sensitivity of cells at higher doses (29).

After a 10-Gy high dose, the transcriptional response included the greater number of genes (245 genes) which were predominantly depressed. These down-regulated genes can be grouped into functional categories: signal transduction, chromatin dynamic and cell cycle, transcription regulator or DNA-banding factor, cytokeleton and cell movement and immunological regulation. Some of these genes were also altered by 2-Gy irradiation (Table IV and V). The detailed lists are available upon request.

In this study, we highlighted the IR-inducible genes whose expression was depressed or increased in a dose-dependent trend from a 0.05-Gy low dose to a 10-Gy high dose (Table IV and Table V). From these inducible genes, the altered expression of *XPC* and *TP53I3* was particularly studied because of its role in global genomic repair (XPC) or the p53 pathway (TP53I3), respectively. Our data are consistent with the observation of a 5-fold increase in the expression of *XPC* in human lymphoid TK6 cells 4 h after 10 Gy (40). IR-induced expression of *XPC* was also shown to depend on the dose rate (26). While it was recently described that the expression level of *XPC* was variable 4 h after irradiation and no distinct pattern was detected, at 24 h XPC expression was variable at the lower doses of 0.5, 1, and 5 Gy in contrast to the robust decrease in expression at the 10- and 20-Gy doses also in TK6 cells (41). Our microarray assay, Northern blot analysis and quantitative real-time PCR data showed a clear dose-dependent induction of the *XPC* gene in AHH-1 cells 4 h after irradiation with doses from 0.05-10 Gy. Our data also indicated that the accumulation of *XPC* mRNA increased in a time-dependent manner, reaching a peak at 4 h and gradually returning to a constitutive level at 10-24 h after exposure to 2 Gy. After a 0.05-Gy low-dose irradiation, the accumulation of *XPC* mRNA reached a peak at 10 h and declined to a constitutive level at 24 h.

It is well known that the p53 tumor suppressor plays a key role in mediating apoptosis and the cell cycle checkpoint by various intracellular and extracellular signals including IR. A number of proteins have been identified to be involved in the p53 pathway, including PCNA, CDKN1A/p21, MDM2, GDF15, TNFRSF10B/TRAIL-R2, TP53I3/PIG3 and GADD45 (42). Among these p53 target genes, *TP53I3* was proposed to participate in the process of p53-dependent apoptosis through reactive oxygen species (ROS) modulation, and its inducible expression was mediated by p53 mainly via a microsatellite (TGYCC)(n) located in its 5'-upstream regulatory region and not through a classical El-Deiry consensus sequence (43,44). Interestingly, an increased expression of *TP53I3* was found in AHH-1 cells 4 h after IR in a clear dose-dependent manner. The biological significance and its mechanism, such as the involvement in apoptosis induction and increased expression of *TP53I3* in response to ionizing radiation warrant further investigation.

Taken together, we identified a series of ionizing radiation-inducible genes, some of which showed altered transcription levels in a clear dose-dependent manner. These transcriptional responses will not only expand our understanding of the mechanisms of cellular responses to ionizing radiation but will also provide candidates for developing potential biomarkers, which can be used to monitor or predict radiation injury.

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