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ORIGINAL CONTRIBUTION

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Gene expression profiles in the liver of mice irradiated with ^{60}Co gamma rays and treated with soybean isoflavone

Abstract *Purpose* To better understand the molecular mechanisms underlying the radio-protective effect of soybean isoflavone that we observed in our recent animal experiments. *Materials and methods* We utilized a cDNA microarray to investigate the expression profiles of 4,096 known genes in the livers of irradiated-mice with or without soybean isoflavone treatment. Dye swap approach was employed to control for gene-specific dye bias and quantitative real-time RT-PCR was performed on several genes to validate the cDNA microarray data. *Results* Compared with the control group, 68 genes were up-regulated and 28 genes were down-regulated in mice treated with irradiation alone, whereas only 6 genes were down-regulated and 35 genes were up-regulated in mice treated with soybean isoflavone. Interestingly, some of the down-regulated genes in the irradiated group, such as DNA repair and stress response genes and cytoskeleton-associated genes, which are markers of cellular damage after irradiation, were maintained at close to normal expression levels after soybean isoflavone treatment. *Conclusions* Comparison of gene expression profiles in the livers of irradiated-mice treated with or without soybean isoflavone suggested that soybean isoflavone may be an efficient tool to reverse irradiation damage of the liver through multiple-pathways and also provides important clues to further pursue the molecular mechanisms underlying the radio-protective activity of soybean isoflavone.

Key words soybean isoflavone – cDNA microarrays – free radicals – irradiation protection

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Abbreviations SI: soybean isoflavone, MAPK: mitogen-activate protein kinase, MUK/DLK/ZPK: Mitogen-activated protein kinase upstream kinase/dual leucine zipper-bearing kinase/leucine-zipper protein kinase, JNK: c-Jun N-terminal kinase, MBIP: MUK binding Inhibitory Protein, HERPUD1: homocysteine-induced endoplasmic reticulum ubiquitin-like domain member 1, HSPA5 (GRP78): heat shock 70 kD protein 5 (glucose-regulated protein), ER: endoplasmic reticulum, Bok: Bcl-2-related ovarian killer protein, Dusp16: dual specificity phosphatase 16, Fdft1: farnesyl diphosphate farnesyl transferase 1, Nfyb: nuclear transcription factor-Y beta, Btg2: B-cell translocation gene 2, anti-proliferative

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Introduction

Radiotherapy is an important treatment strategy for cancer patients. However, irradiation can indirectly activate H_2O and yield a large amount of excessive free radicals [1], breaking the balance between the oxidant and antioxidant systems and leading to oxidative stress in the cell. Oxidative damage to normal cells and organs can cause serious adverse reactions such as nausea, vomiting, and weakness of immunological function, which makes some patients give up therapy. Over the years, a number of natural compounds such as polysaccharides [2], vitamin E [3], and flavonoids [4, 5], as well as artificial compounds such as dipyridamole [6, 7] have been tested for their radioprotective efficacy with generally limited success.

Recently, soybean isoflavone (SI) has drawn wide attention due to its potentially efficacious effects on some human degenerative diseases. Data from animals and in vitro studies suggest that SI or soybean diets are beneficial in reducing the incidence of cardiovascular disease and osteoporosis, as well as inhibiting radiation- and carcinogen-induced tumors of various tissues [8–12]. In particular, preliminary studies indicated that genistein and daidzein, the two main active compounds of SI, possess antioxidant activity both in vivo and in vitro. Intake of genistein and daidzein provides protection against oxidative modification of low-density lipoprotein particles in human volunteers [13]. Importantly, Wei H et al. [14–19] demonstrated that genistein significantly inhibits ultraviolet (UV) light-induced oxidative DNA damage in purified DNA and cultured cells, and blocks UVB-induced c-fos and c-jun proto-oncogene expression in mouse skin. They also found that genistein blocks UVB-induced phosphorylation of epidermal growth factor receptor and mitogen-activated protein kinase (MAPK) activation in vitro [5]. Our previous study found that administration of SI at different doses (50, 100, and 400 mg/kg b.wt) significantly elevated both enzyme activity and mRNA levels of catalase and glutathione peroxidase, and decreased malonaldehyde levels in the liver. SI treatment also accelerated the recovery of WBC and RET at day 7 after irradiation. The best effect was seen with an intermediate dose of SI (unpublished data). Although these data indicated a protective role for SI against irradiation, the cellular and molecular mechanisms are still unclear. A comprehensive understanding of the molecular biological properties of SI may be useful in developing strategies to reduce radiotherapeutic adverse reactions.

In recent years, cDNA microarrays have been used to study genistein's estrogen bioactivity and anticancer activity [20, 21]. In addition, the gene expression profiles of human cells exposed to UV and ionizing radiation [22, 23] and the alteration of gene expression profiles by other irradiation-protective agents have been studied [24]. However, little is known about the gene expression profiles of liver tissue subjected to irradiation after SI treatment. To better understand the precise molecular mechanisms underlying the radioprotective effect of SI that was shown in our recent animal experiments, we utilized a cDNA microarray to investigate the mRNA levels of 4,096 genes in liver tissues treated with irradiation alone (IR) or with irradiation plus SI. Comparison of the gene expression profiles from these two groups suggested that administration of SI may be an efficient means to mitigate irradiation damage of the liver through multiple pathways, and also provided important clues to further investigate the molecular mechanisms underling the radioprotective activity of SI.

Materials and methods

Animals, reagents, and irradiation

Female Swiss albino mice were purchased from the Center of Laboratory Animals, Second Military Medical University (Shanghai, China). Animals were 10-weeks-old and weighed between 23 and 29 g at the beginning of the experiment. They were fed with standard mice pellet food and water and kept at a temperature of 25 ± 1°C, a relative humidity of 50 ± 5%, and with ventilation amounting to 15 air renewal cycles/h in a light-dark cycle for 24 h for one week before administration of drugs. The research protocol and all animal experiments received prior permission and followed the guidelines of the ethics committee of the Second Military Medical University.

A 41.56% SI extract (containing isoflavone daidzin 16.42%, daidzein 1.03%, genistin 22.63%, genistein 1.48%) was provided by LuFeng Bio-Products Company Ltd. (Dalian, China). The 1.15% suspensions were prepared with corn oil and mixed vigorously prior to use.

For in vivo experiments, irradiation was performed with ^{60}Co-γ rays in the actinotheraphy department of Changhai hospital. The mice were placed in Perspex-covered boxes and exposed to 4.56 Gy at a rate of 0.57 Gy/min.

Treatment methods

Eighteen mice were randomized into three groups based on body weight: the SI treatment + irradiation

group (SI group), the irradiation alone group (IR group), and the control group (C group). Mice in the C and IR groups received corn oil, while mice in the SI group received 100 mg/kg b.wt SI daily for 14 consecutive days. For whole-body irradiation experiments, mice from the IR and SI intervention groups were given 4.56 Gy of ^{60}Co-γ irradiation once on the 7th day after treatment and killed by cervical dislocation on the 2nd day after irradiation. The entire liver was removed from each animal, immediately frozen in liquid N_2, and stored at −80°C until RNA extraction.

Microarray sample preparation, probe labeling, and hybridization

The cDNA microarrays were provided by Biostar Genechip Inc., Shanghai, China. The BioStar M-40s microarray consists of 4,096 novel or known genes including control system and function genes. The control system consists of 96 housekeeping genes as loading controls, as well as 16 plant genes and spotting solution (without DNA, 16 spots) as negative controls.

The cDNA microarray was performed on paired samples: Irradiation samples are referred to the controls, and irradiation + SI treatment samples are also referred to controls. In brief, total RNA was prepared from livers using Trizol reagent (Invitrogen, Carlsbad, CA), and the quality assessed by absorbance at 260/280 nm as well as by electrophoresis in 1% agarose gels by staining of the 28S rRNA with ethidium bromide.

Equal amounts of RNA from six mice from each group were mixed. The fluorescent cDNA probes were prepared through reverse transcription with Cy3- or Cy5-deoxy UTP (Amersham Phamacia Biotech, Piscataway, NJ) as follows: 5 μg of oligo(dT) 18 and 50 μg of total RNA were incubated at 70°C for 10 min, and then chilled on ice. The reaction was carried out in a mixture containing dNTPs (200 μM dATP, dCTP, dGTP, 60 μM dTTP, and 60 μM Cy3- or Cy5-dUTP), 2 μl of Superscript II reverse transcriptase (Invitrogen, USA), and 1× reaction buffer at 42°C for 2 h. The RNA was then hydrolyzed by addition of 4 μl of 2.5 M NaOH and incubation at 65°C for 10 min, followed by neutralization with 4 μl of 2.5 M HCl. To control for gene-specific dye bias in a direct comparison design, a dye swap approach was employed on replicate microarrays. That is, the RNA isolated from each group was separated into two aliquots, one labeled with Cy5-dUTP and the other with Cy3-dUTP. Each of these samples was co-hybridized on a microarray with the reference sample labeled by the alternative dye. After quantification of fluorescence, the labeled probes were mixed and diluted in 500 μl Tris–EDTA and concentrated to 10 μl with a Microcon YM-30 filter (Millipore, Bedford, MA). Labeled probes were purified and dissolved in 20 μl of hybridization solution (5× SSC, 0.75 M NaCl and 0.075 M sodium citrate) with 0.4% SDS and 50% formamide. cDNA microarrays were pre-hybridized with hybridization solution containing 0.5 mg/ml denatured salmon sperm DNA at 42°C for 6 h. Fluorescent probes were denatured at 95°C for 5 min and applied to the pre-hybridized chips under a glass coverslip. Chips were hybridized at 42°C for 15–17 h. After hybridization, the chips were washed at 60°C for 10 min each in solutions of 2× SSC and 0.2% SDS, 0.1× SSC and 0.2% SDS, and 0.1× SSC, then dried at room temperature.

Chip scanning and data analysis

Hybridized chips were scanned with a ScanArray 4000 (GSI Lumonics, Billerica, MA) at 532 and 653 nm to detect emission from Cy3 and Cy5, respectively. The resulting images were analyzed using GenePix Pro 3 software (Axon Instruments Inc., USA). The intensities of each spot at the two wavelengths represented the quantity of Cy3-dUTP and Cy5-dUTP, and the ratios of Cy5/Cy3 were computed using the GenePix Pro 3 median of ratio method. Overall intensities were normalized using the corresponding GenePix default normalization factor. All spots flagged 'Bad' or 'Not Found' by the GenePix software were removed from the final data set. To minimize artifacts from low expression values, only genes with raw intensity values >200 counts for both Cy3 and Cy5 or >800 counts for one of them were chosen for further analysis. Genes were identified as differentially expressed if the relative fluorescence intensities (Cy5/Cy3) were greater than 2 or less than 0.5.

Validation of gene expression data by quantitative real-time RT-PCR

Quantitative real-time RT-PCR of selected genes was used to validate data from the cDNA microarrays. In brief, total RNA was extracted from liver samples of each mouse using Trizol Reagent (Life Technologies Inc, Rockville, MD, USA), according to the protocol recommended by the manufacturer. Two micrograms of total RNA was subjected to an RT reaction using Superscript II Reverse Transcriptase in 25 μl of reaction mixture (Invitrogen). Quantitative real-time PCR was performed in a Light Cycler System using the Fast Start DNA master SYBR green I kit as recommended by the manufacturer (Roche, Mannheim, Germany). In a total volume of 20 μl, each reaction contained 2 μl SYBR green I reaction mix (consisting

of Taq DNA polymerase reaction buffer, dNTP mix, SYBR green I, $MgCl_2$ and Taq DNA polymerase), 0.4 μM of each primer, 4 mM $MgCl_2$ and 2 μl cDNA, nuclease-free water as a negative control. Threshold values were assigned where the normalized reporter signal Δ(Rn)-to-noise ratio exceeded 1.0 for all genes in the plot. The midpoint of the linear phase of exponential amplification was determined to be the threshold cycle (Ct) for each gene. To determine relative expression levels in each mRNA population, a standard curve was plotted based on expressions of GAPDH in dilutions of RNA from the control group. After each run, melting curve analysis was performed to verify the specificity of the PCR reaction. Each sample was run and analyzed in triplicate. For all experimental samples, the amount of product was determined from the standard curve, averaged, and divided by the average amount of GAPDH product to achieve a normalized value. Sequences of the primers used are shown in Table 1.

Statistical analysis

Real-time RT-PCR results were expressed as the mean ± SD and a two-tailed Student's *t* test was used. Differences were considered statistically significant when *P* values were <0.05.

Results

We observed a broad cellular response in the liver after irradiation alone and irradiation plus SI treatment by our cDNA microarray. Dye swap was conducted on a single mRNA preparation; thus, each sample was performed in quadruplicate (duplicate experiments with each fluorescent label to account for dye effects). As shown in Fig. 1A, most of the differentially expressed genes (yellow spots) were under the diagonal when the IR samples were labeled with Cy5 and the reference sample with Cy3. On the contrary, most of the yellow spots were scattered upward when the samples were labeled with the opposite dye orientation; and we also found that the normalized ratio of nearly 90% of the loading control spots was around 1 and the negative control spots showed low intensity after hybridization, confirming the reliability of these experiments.

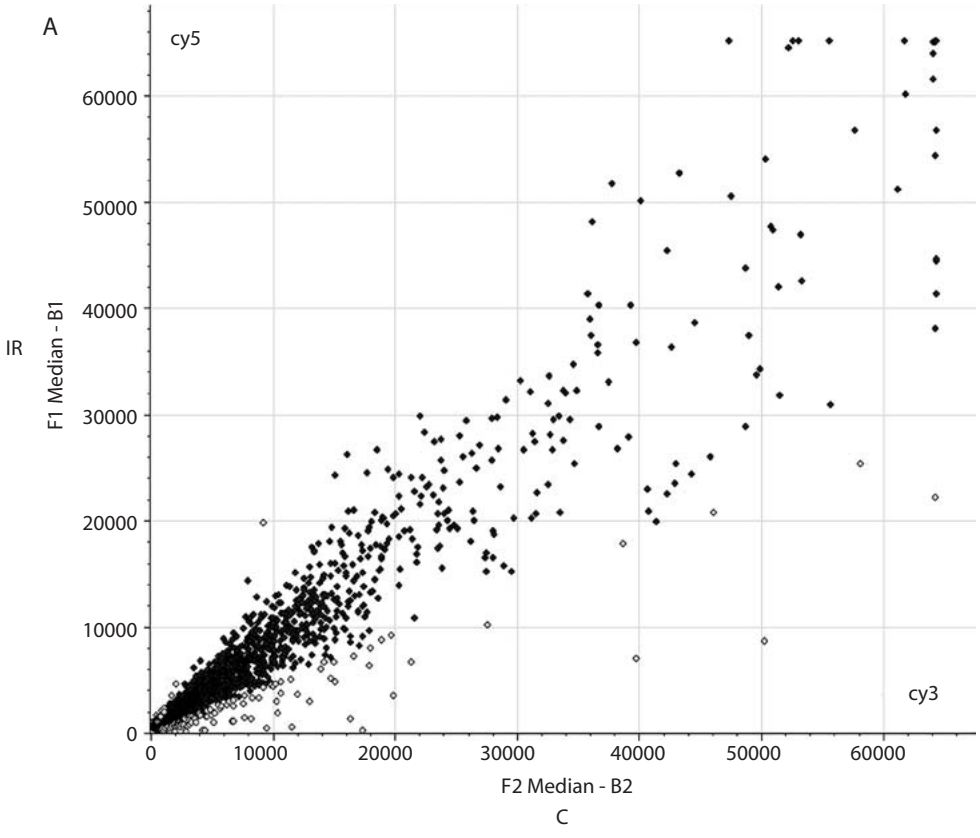
Functions of the differentially expressed genes were analyzed by Gene Ontology (http://www.geneontology.org) and Medline database searches (Fig. 2). On the basis of Gene Ontology descriptions for gene products, we classified these up- and down-regulated genes into six categories: DNA repair and stress response, cytoskeleton, signaling transduction, metabolism, hydronium channel/transport, and immune related proteins. In the IR/C chip, a total of 96 genes were differentially expressed with a threshold of a 2.0-fold change, with 68 genes down-regulated and 28 genes up-regulated. Parts of genes with identified functions are listed in Table 2. The majority of the down-regulated genes were involved in the cytoskeleton (8), signal transduction (6) and DNA repair and stress response (7); whereas the up-regulated genes were mainly involved in signal transduction (3), metabolism (3), DNA repair and stress response (2) and cytoskeleton (2).

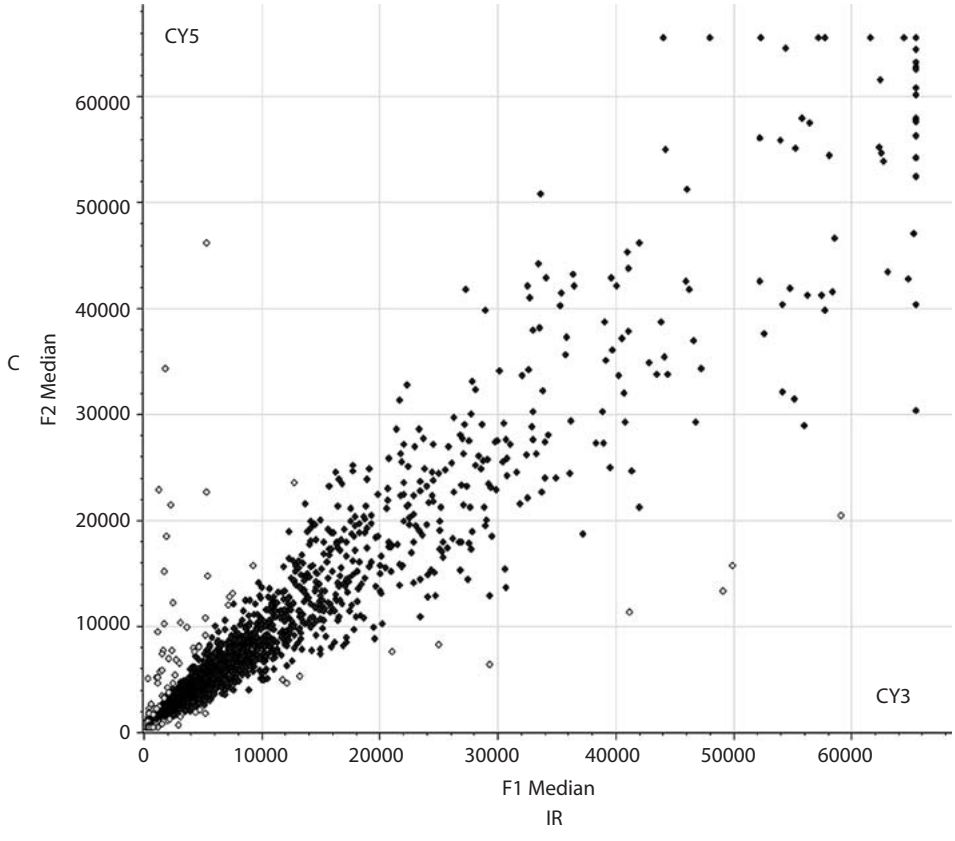
Interestingly, after SI treatment, the total number and categories of altered genes, and the proportion of activated and repressed genes were significantly different compared with the IR group (Fig. 1, Table 3). A total of 41 genes were differentially expressed in the SI/C chip, much less than that the 96 genes differentially expressed in the IR/C chip. Among these, 35 genes were up-regulated and 6 genes were down-regulated. Up-

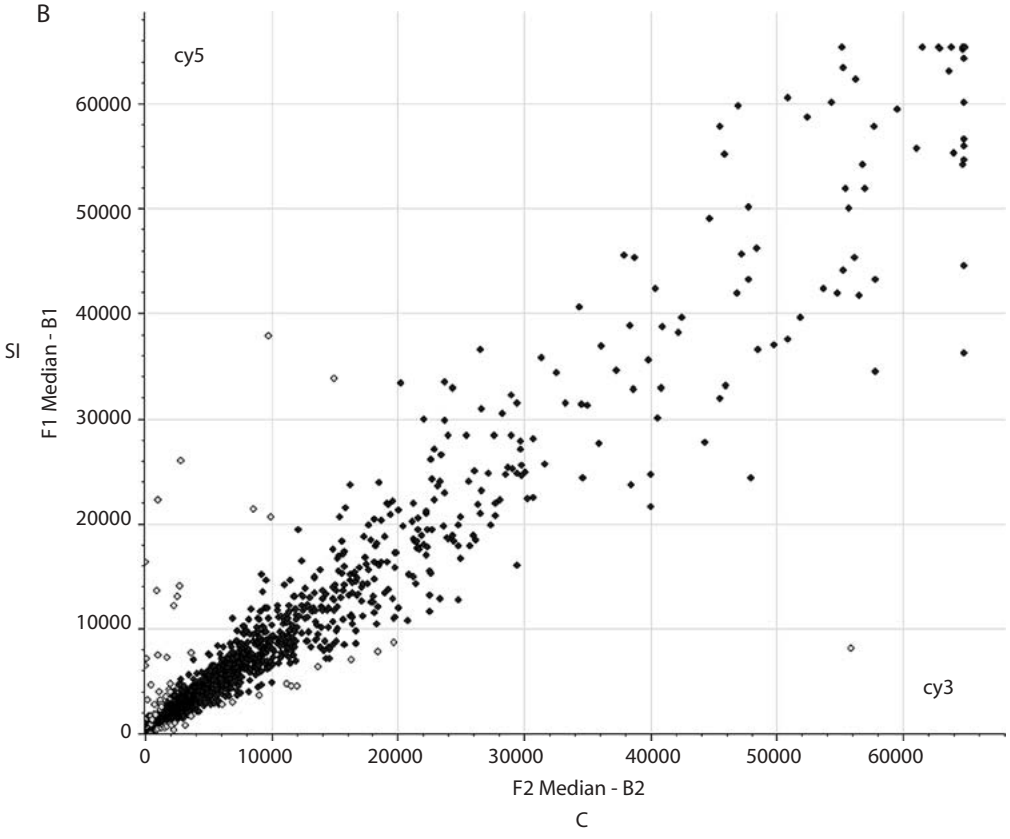
Fig. 1 Scatter plots of cDNA microarrays of liver tissue from different groups. (**A**) IR group *vs.* control group, (**B**) SI group versus control group. Differential genes appear as open spots (fold change <0.5 or >2) and unaffected genes appear as black spots (0.5 < fold change < 2). IRcy5/Ccy3 indicates values where the IR samples were labeled with Cy5 and the reference sample with Cy3. IRcy3/Ccy5 represents the values for the opposite dye orientation. Induced genes are visualized as a shift upward from the diagonal, while suppressed genes are shifted downward

Table 1 Sequences of the primers used for mRNA quantization by real-time RT-PCR

Gene	Sense primers	Anti-sense primers	bp
GAPDH	5′-AACGACCCCTTCATTGAC-3′	5′-TCCACGACATACTCAGCAC-3′	191
HSPA5(GRP78)	5′-AACCAACTCACGTCCAACCC-3′	5′-TGCCCACCTCCAATATCAAC-3′	164
Btg2	5′-TGAAGTGTCTTACCGCATCG-3′	5′-GGTGGCTCCTATCTAGCTGGAGACG-3′	152
Dusp16	5′-ACTGATGAAGCGAAGGTTGC-3′	5′-TACCCAGAAGTACAGTGAGAAAGC-3′	173
Nfyb	5′-CACAGATGGACTAAGCGAAGA-3′	5′-GTTCCGTCCGTCCTTCCGATCATGA-3′	147
HERPUD1	5′-GTGGAGGAAGATGATGAGATAAA-3′	5′-CGTGGTGCAGGTACATGACTA-3′	154
MBIP	5′-TTGTGGTAATCAGGCGGTAG-3′	5′-GTTCTGTTGAGGTGGTTGGA-3′	208
Fdft1	5′-CATAACCAACACCCTACAGC-3′	5′-AGGGTGACTGCTTGCCCCTTCCGAA-3′	169
Bok	5′-CCACATCTTCTCAGCAGGTATCAC-3′	5′-TCTTGCGTACAAACTCCCCCAGGCA-3′	152



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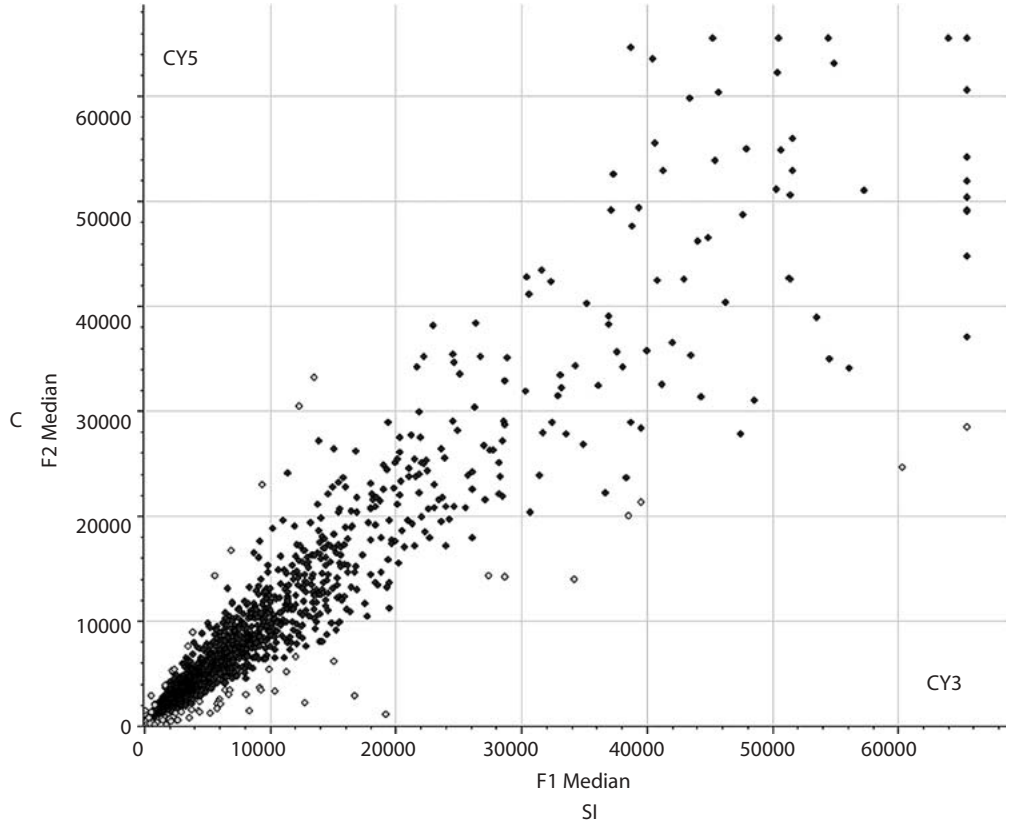
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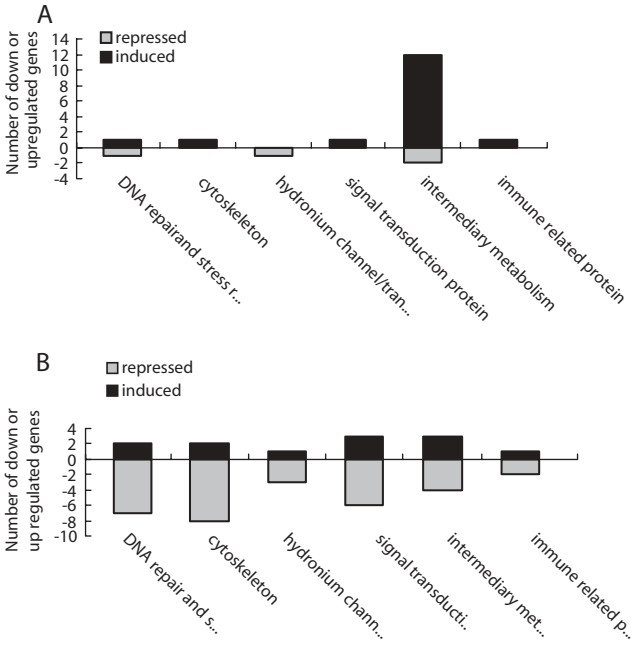

Fig. 2 Functional categories and numbers of differentially expressed genes. Gene function was determined by Medline database searches and classified into six categories. (**A**) IR group versus control group, (**B**) SI group versus control group

regulated genes were mainly involved in metabolism (12), DNA repair and stress response (1), cytoskeleton (1) and cell signaling (1), while the down-regulated genes were mainly involved in metabolism (2).

In comparing the two chips, we noticed that expression of most of the genes up- and down-regulated after irradiation did not change after SI treatment. For example, B-cell translocation gene 2 (NM_007570), a protein that negatively regulates apoptosis and the response to DNA damage, was down-regulated in the IR/C chip, but kept at normal levels after SI treatment.

To evaluate the reliability of the cDNA microarray results, expression of a subset of up-regulated genes, which included Nfyb (NM_010914), HERPUD1 (NM_022331), MBIP (NM_145442) and Fdft1 (NM_010191), and a subset of down-regulated genes, which included HSPA5 (GRP78) (NM_022310), Btg2 (NM_007570), Dusp16 (NM_130447) and Bok (NM_016778), was validated by quantitative real-time RT-PCR. The results are shown in Fig. 3. Compared with the control group, expression of HSPA5, Btg2, Dusp16 were significantly decreased ($P < 0.01$, $P < 0.01$, $P < 0.01$, respectively) and Nfyb was significantly induced ($P < 0.01$) after irradiation, but did not change significantly after SI treatment; HERPUD1, MBIP were up-regulated ($P < 0.01$, $P < 0.01$, respectively), and Bok was down-regulated ($P < 0.01$) in the SI group, but the two genes have no significant change in IR group; Fdft1 was up-regulated in both the SI ($P < 0.01$) and IR groups ($P < 0.01$). Although no difference of Dusp 16 was observed on the SI/C chip, and no difference of MBIP were observed on the IR/C chip, real-time PCR results indicated that these genes were significantly induced ($P < 0.05$ vs. C group). Thus, among the 16 values (eight genes) examined, fourteen paralleled the results obtained with cDNA microarray.

Discussion

The gene expression profiles showed that the cellular response to irradiation involves the induction of some genes and the repression of other genes, which play important roles in various biochemical and regulatory pathways. Among the down-regulated genes in the IR group, cytoskeletal and hydronium channel/transport proteins accounted for the largest proportion. Repression of these genes suggested that a large amount of reactive oxygen species produced by irradiation destroyed the cell cytoskeleton and membrane structure and led to abnormal transport. Other noteworthy suppressed genes were components of the Ras signal transduction pathway and its downstream component, ribosome protein S6 kinase. The Ras/Raf/MAPK is one of the important signal transduction pathways in irradiation, and previous studies have indicated that the elevation of ras gene expression could increase the irradiation resistance of the cell [25–27]. Repression of these genes suggested that the cellular resistance to irradiation decreased. In addition, down-regulation of immune-related proteins leads to the impairment of immune function; some of metabolism genes were also down-regulated, suggesting that metabolic processes had become disorganized.

Besides the repressed genes in the IR group mentioned above, irradiation activated some genes that have important functions in controlling cell division and the cell cycle. For example, cytoplasmic dynein intermediate chain is a necessary component in preserving the function of dynein, which is responsible for nuclear migration during mitosis [28], and its over-expression after irradiation may lead to the perturbation of mitosis. The activation of the CDC28 protein kinase regulatory subunit, nuclear transcription factor, and PRP4 kinase were probably responsible for abnormalities in cell cycle control, transcription, and translation.

Compared with the IR/C chip, the numbers of differentially expressed genes in the SI/Cchip were reduced and the proportion of up-regulated genes was larger than that of down-regulated genes. Metabolism-related genes accounted for the largest propor-

Table 2 Down- and Up-regulated genes in liver of IR group as compared to C group[a]

Gene ID	Gene function [b]	Mean fluorescence intensity ratio, Cy5/Cy3[c]			
		IR/C		SI/C	
		$IR_{(Cy5)}$,$C_{(Cy3)}$	$IR_{(Cy3)}$,$C_{(Cy5)}$	$SI_{(Cy5)}$,$C_{(Cy3)}$	$SI_{(Cy3)}$,$C_{(Cy5)}$
Down-regulated genes					
DNA repair and stress response					
NM_011803	core promoter element binding protein (Copeb)	0.346	5.448	1.979	0.927
NM_008211	H3 histone, family 3B (H3f3b)	0.452	2.512	0.739	1.163
NM_011580	thrombospondin1 (Thbs1)	0.172	5.718	0.598	1.701
NM_022310	heat shock 70kD protein 5 (glucose-regulated protein)(Hspa5)	0.404	2.220	1.026	1.044
NM_007570	B-cell translocation gene 2, anti-proliferative (Btg2)	0.208	2.155	1.091	0.991
NM_007630	cyclin B2 (Ccnb2)	0.054	6.418	0.721	0.857
NM_130447	dual specificity phosphatase 16 (Dusp16)	0.477	2.594	0.686	0.847
Cytoskeleton					
NM_013615	outer dense fiber of sperm tails 2 (Odf2)	0.241	2.061	1.189	0.952
NM_008470	keratin complex 1, acidic, gene 16 (Krt1-16)	0.366	2.833	0.547	1.715
NM_008475	keratin complex 2, basic, gene 4 (Krt2-4)	0.304	2.388	0.821	1.541
NM_010129	epithelial membrane protein 3 (Emp3)	0.432	2.528	1.148	0.756
NM_007393	actin, beta, cytoplasmic (Actb)	0.177	3.296	0.960	1.340
NM_016845	proacrosin binding protein (Acrbp)	0.209	3.624	0.866	0.939
NM_024427	tropomyosin1, alpha (Tpm1)	0.394	2.306	0.913	0.856
NM_009898	Coronin, actin binding protein 1A (Coro1a)	0.462	2.318	0.924	0.821
Hydronium channel /Transport protein					
NM_134086	solute carrier family 38, member 1 (Slc38a1)	0.221	3.250	0.911	1.130
NM_013667	solute carrier family 22 (organic cation transporter), member 2 (Slc22a2)	0.269	2.954	0.750	1.590
NM_008880	phospholipid scramblase 2 (Plscr2)	0.386	3.300	0.563	1.808
Signal transduction protein					
NM_028035	sorting nexin 10 (Snx10)	0.487	3.632	1.129	0.936
NM_011777	zyxin (Zyx)	0.355	4.326	1.156	1.246
NM_021485	ribosomal protein S6 kinase, polypeptide 2 (Rps6kb2)	0.210	3.021	0.795	0.826
NM_007484	ras homolog (RhoC) gene family, member C (Arhc)	0.407	2.515	0.802	1.523
NM_009025	RAS p21 protein activator 3 (Rasa3)	0.374	2.894	0.853	1.115
NM_010937	neuroblastoma ras oncogene (N-ras)	0.497	2.852	1.251	1.362
Intermediary metabolism					
NM_008807	tubby-like protein 2 (Tulp2)	0.271	2.157	1.812	1.692
NM_021528	Chondroitin4-sulfotransferase12 (C4st2-pengding)	0.116	6.703	0.649	0.890
NM_008492	lactate dehydrogenase2, B chain (Ldh2)	0.466	4.532	1.820	1.313
NM_017372	lysozyme (Lyzs)	0.289	3.208	0.471	1.110
Immune related protein					
U68543	Ig single-chain antibody	0.237	3.766	0.650	0.736
NM_010260	guanylate nucleotide binding protein 2 (Gbp2)	0.351	2.961	1.172	0.687
Up-regulated genes					
DNA repair and stress response					
NM_025415	CDC28 protein kinase regulatory subunit 2 (Cks2)	2.555	0.487	0.833	0.924
U39818	tuberous sclerosis 2 (Tsc2)	2.682	0.344	1.122	1.515
cytoskeleton					
NM_010064	dynein, cytoplasmic, intermediate chain 2 (Dncic2)	2.206	0.392	1.601	0.753
BC008140	tektin 2 (Tekt2)	2.300	0.432	1.240	0.790
Hydronium channel /Transport protein					
NM_018815	nucleoporin 210 (Nup210)	2.968	0.401	0.935	0.739
Signal transduction protein					
NM_010914	nuclear transcription factor-Y beta (Nfyb)	2.822	0.358	0.783	1.025
AF283466	PRP4 kinase (Prp4k)	2.168	0.424	0.755	0.912
NM_011100	protein kinase, cAMP dependent, catalytic, beta (Prkacb)	3.046	0.478	1.282	0.939
Intermediary metabolism					
NM_010274	glycerol phosphate dehydrogenase 2, mitochondrial (Gpd2)	2.205	0.495	1.214	2.053
NM_010191	Farnesyl diphosphate farnesyl transferase 1 (Fdft1)	2.269	0.475	2.562	0.436
NM_008706	NAD(P)H dehydrogenase, quinone 1 (Nqo1)	4.118	0.204	1.330	0.959
Immune related protein					
NM_021718	lymphocyte antigen 116 (Ly116)	3.790	0.226	0.893	0.837

Note: [a]The corresponding results for the SI/C chip are also listed. [b]Gene function was defined by Medline database searches and analyzed using Gene Ontology (GO) (http://www.geneontology.org); [c]In each chip, dye swap approach was employed and duplicate experiments were performed with each fluorescent label. Genes were identified as differentially expressed if the relative fluorescence intensities, Cy5/Cy3, were all greater than 2 or less than 0.5 in the four hybridization experiments. Data represent the mean relative dye intensities (Cy5/Cy3) of two duplicate experiments with the same fluorescent label

Table 3 Down- and up-regulated genes in liver of SI group as compared to C group[a]

Gene ID	Gene function [b]	Mean fluorescence intensity ratio, Cy5/Cy3 [c]			
		SI/C		IR/C	
		$SI_{(Cy5)}$,$C_{(Cy3)}$	$SI_{(Cy3)}$,$C_{(Cy5)}$	$IR_{(Cy5)}$,$C_{(Cy3)}$	$IR_{(Cy3)}$,$C_{(Cy5)}$
Up-regulated genes					
DNA repair and stress response					
NM_022331	homocysteine-induced endoplasmic reticulum ubiquitin-like domain member 1 (Herpud1)	2.337	0.397	0.568	0.945
cytoskeleton					
J04695	Procollagen, alpha-2 type IV	5.093	0.306	0.817	1.722
Signal transduction protein					
NM_145442	Mus musculus MAP3K12 binding inhibitory protein 1(MBIP)	3.505	0.423	1.523	1.006
Intermediary metabolism					
NM_026419	elastase 3B, pancreatic (Ela3b)	18.386	0.232	1.272	1.221
NM_018874	pancreatic lipase related protein 1 (Pnliprp1)	26.735	0.441	1.000	0.924
NM_009430	trypsin 2 (Try2)	31.23	0.044	1.167	1.024
NM_007919	elastase 2 (Ela2)	81.61	0.044	1.513	0.856
NM_009669	amylase 2, pancreatic (Amy2)	4.319	0.094	1.082	1.111
NM_009981	phosphate cytidylyltransferase 1, choline, alpha isoform (Pcyt1a)	6.995	0.255	1.145	0.787
NM_010294	glucokinase activity, related sequence 2 (Gk-rs2)	3.087	0.488	1.130	1.087
NM_023182	chymotrypsin-like (Ctrl),	14.325	0.181	1.468	0.975
NM_019741	solute carrier family 2 (facilitated glucose transporter)	2.971	0.185	0.583	2.730
NM_010191	farnesyl diphosphate farnesyl transferase 1 (Fdft1)	2.562	0.436	2.269	0.475
NM_011271	ribonuclease, Rnase A family, 1 (pancreatic) (Rnase1)	3.999	0.249	0.860	1.078
NM_007643	CD36 antigen (Cd36)	2.462	0.384	0.841	1.373
Immune related protein					
NM_175217	Mus musculus monocyte to macrophage differentiation-associated 2 (Mmd2)	2.262	0.457	1.121	1.098
Down-regulated genes					
DNA repair and stress response					
NM_016778	Bcl-2-related ovarian killer protein (Bok)	0.437	3.025	1.000	1.155
Hydronium channel/Transport protein					
NM_008536	transmembrane 4 superfamily member 1 (Tm4sf1)	0.442	2.721	0.626	1.625
Intermediary metabolism					
U27014	sorbitol dehydrogenase precursor	0.264	3.156	0.465	0.806
NM_017372	lysozyme (Lyzs)	0.471	2.539	0.289	3.208

Note: [a]The corresponding results for the IR/C chip are also listed. [b]Gene function was defined by Medline database searches and analyzed using Gene Ontology (GO) (http://www.geneontology.org); [c]In each chip, dye swap approach was employed and duplicate experiments were performed with each fluorescent label. Genes were identified as differentially expressed if the relative fluorescence intensities, Cy5/Cy3, were all greater than 2 or less than 0.5 in the four hybridization experiments. Data represent the mean relative dye intensities (Cy5/Cy3) of two duplicate experiments with the same fluorescent label

tion of up-regulated genes in the SI group, which may be a direct effect of SI on metabolism [29–31]. Interestingly, most biomarkers of cellular damage after irradiation, such as cytoskeletal genes and hydronium channel/transport genes, were expressed at close to normal levels in the SI group. For example, the ratios of NM_010129 (epithelial membrane protein) and NM_134086 (solute carrier family 38, member 1) were 0.432 and 0.221 in the IR/C chip, while they were 1.148 and 0.911 in the SI/C, respectively. The expression level of the important signal transduction mediators, NM_007484 (ras homolog (RhoC) gene family, member C (Arhc)) and NM_009025 (RAS p21 protein activator 3 (Rasa3)), which reflect cellular radiation resistance, were also down-regulated in the IR group and close to normal levels after SI treatment (the ratios were 0.407 and 0.374 in the IR/C chip and 0.802 and 0.853 in the SI/C chip). These results suggested that SI might exert its protective effects through multiple physiological pathways and provided new insights to further investigations.

Recent evidence suggests that activation of the JNK/c-Jun pathway plays an important role in triggering apoptosis induced by ionizing radiation [32–34] and that inhibition of JNK or c-Jun activity is sufficient to prevent apoptosis [35]. Interestingly, we found that MBIP and HERPUD1, two proteins able to inhibit the activation of the JNK/c-Jun pathway, were both significantly up-regulated after SI treatment. MBIP contains two tandem orientated leucine-zipper-like motifs with a cluster of basic amino acids located between the two motifs, overexpression of MBIP could partially inhibit the activation of JNK(c-Jun N-terminal kinase) by inhibiting the ability of MUK/DLK/ZPK [36]. Mouse HERPUD1 is the

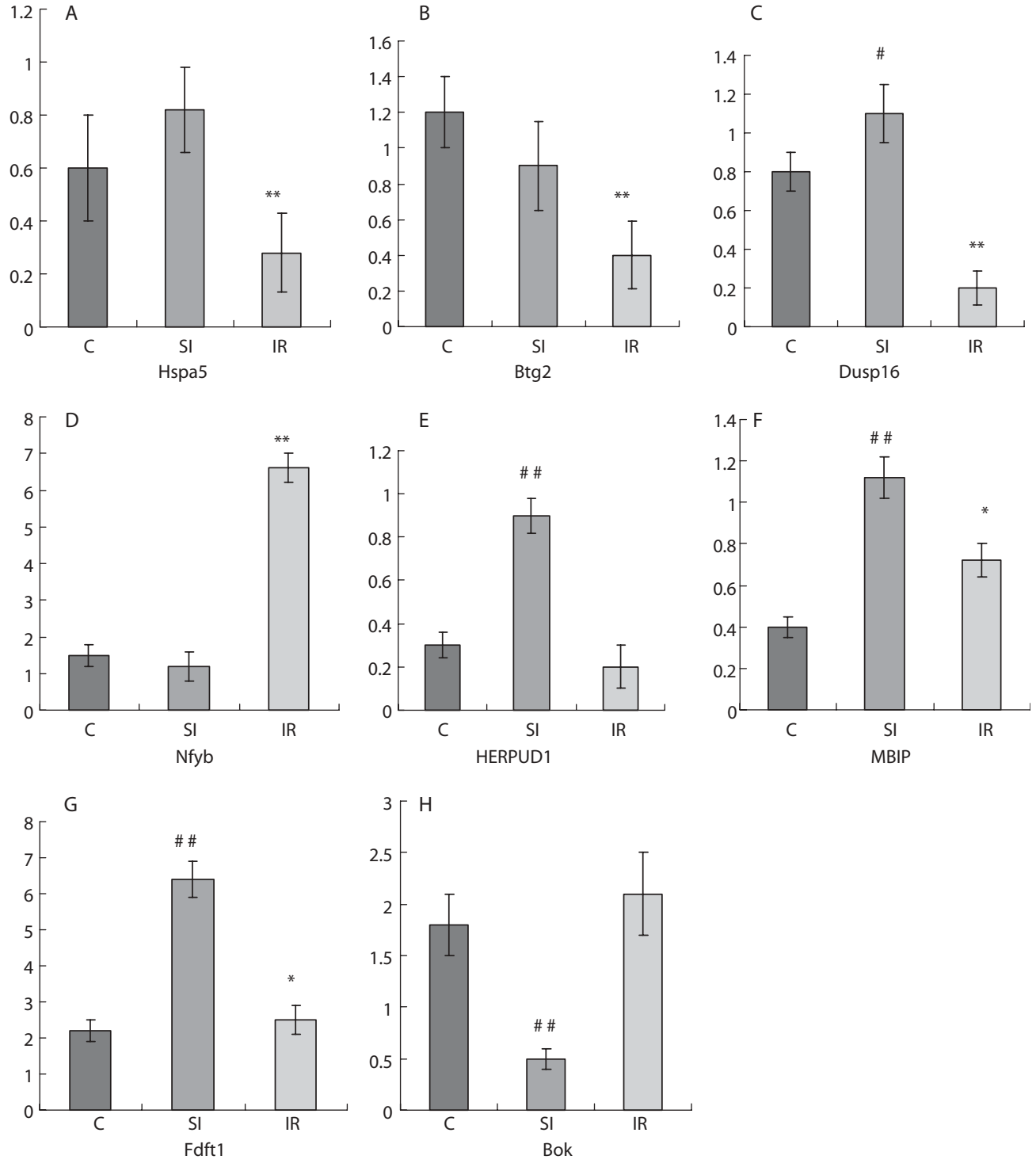

Fig. 3 Validation of expression of selected genes by quantitative real-time RT-PCR. Analyses were performed in triplicate for each mouse. GAPDH was included as an internal control. (**A**–**H**) Mean relative mRNA population of selected gene *vs.* GAPDH in IR, SI, and C groups. HSPA5 (GRP78), Btg2, Dusp16 are down-regulated genes in IR/C chips; Nfyb is up-regulated gene in IR/C chip; HERPUD1, MBIP, Fdft1 are down-regulated genes in SI/C chips; Bok is up-regulated gene in SI/C chips. $^{*}P < 0.05$, $^{**}P < 0.01$, versus control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus IR group

homologue of human Herp (homocysteine-induced ER protein). Herp is a novel 54 kDa protein recently described as a stress-response protein localized in the ER membrane [37]. It was suggested that Herp plays a protective role in brain by stabilizing Ca^{2+} homeostasis and mitochondrial function [38]. Overexpression of Herp was able to reduce c-JUN phosphorylation and prevent apoptosis triggered by the JNK/c-Jun pathway. Therefore, the up-regulation of MBIP and HERPUD1 after SI treatment may inhibit the cell's apoptosis caused by irradiation through the suppression of JNK. Another notable gene is heat shock 70 kD protein 5 (Hspa5), also known as glucose-regulated protein 78 (GRP78), a molecular chaperone that belongs to the highly conserved heat shock protein 70 family. It was repressed after irradiation but maintained at normal levels after SI treatment. GRP78 is constitutively expressed in the endoplasmic reticulum of mammalian cells [39], but its expression increases in response to various DNA-damaging stresses such as X-ray irradiation and genotoxic chemical compounds, protecting cells from death. Many reports have also indicated that overexpression of GRP78 protects against DNA-damaging stress-induced death in mammalian cells [40–42]. Zhai et al. down-regulated GRP78 in human RSa cells by transfection with antisense cDNA for GRP78 and found that the transfected cells showed a higher sensitivity to UVC-induced cell death and had a lower DNA repair activity. Furthermore, down-regulated expression of GRP78 decreased the expression of other glucose-regulated proteins such as GRP94, which in turn further increased the cells' sensitivity to irradiation [43]. Therefore, in our present study, the repression of GRP78 after irradiation may lead to loss of its protective activity against DNA damage and increase sensitivity to irradiation-inducted cell death. Maintaining the normal expression of GRP78 after SI treatment may be one key mechanism in protecting against the DNA damage and cell death caused by irradiation.

In conclusion, SI altered the expression of some important genes involved in apoptosis, signal transduction, and redox status in irradiated mouse liver, suggesting that SI is a complex compound and its protective mechanisms against irradiation are likely to be mediated by a variety of regulatory pathways. Comparison of the genes modulated after irradiation with that of modulated after SI treatment may be useful in our understanding of the molecular basis of radiotherapy and in developing strategies to reduce its adverse reaction.

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