ONCOLOGY REPORTS 15: 33-48, 2006

Temporal cDNA microarray analysis of gene expression in human hepatocellular carcinoma upon radiation exposure

JAEMIN JEONG[1,6], SU-JIN HONG[1], YEUN-JIN JU[1], BU-YEO KIM[1], MYUNG-JIN PARK[1],
TAE-HWAN KIM[5], CHUL-IN PARK[1], KANG-YELL CHOI[2], MYUNG-HAENG CHO[3],
SANG-HOON KIM[4], HANSOO LEE[6] and KEE-HO LEE[1]

[1]Laboratory of Molecular Oncology, Korea Institute of Radiological and Medical Sciences; [2]Department of Biotechnology, College of Engineering, Yonsei University; [3]Laboratory of Toxicology, College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University; [4]Department of Biology, Research Institute for Basic Sciences, Kyung Hee University, Hoegi-dong, Dongdaemoon-gu, Seoul; [5]College of Veterinary Medicine, Kyungpook National University, Daegu; [6]Vascular System Research Center, Division of Life Sciences, College of Natural Sciences, Kangwon National University, Chunchon, Korea

Received May 31, 2005; Accepted July 14, 2005

Abstract. Radiotherapy is not commonly used for the treatment of human hepatocellular carcinoma, due to its poor response rate and poor tolerance of normal liver to ionizing radiation. Recently developed microarray technology makes it possible to verify genes responsive to anticancer therapy of human cancers by simultaneous analysis of gene expression profiles. In the present study, the expression profile of radiation-responsive genes in human hepatocellular carcinoma was evaluated through time-dependent cDNA microarray analysis of expressional variation, following exposure to ionizing radiation. Upon exposure to radiation, more than 13% of genes in both radiation-resistant and -sensitive cells responded to radiation. Time-dependent analysis of radiation-responsive genes revealed that, irrespective of radiation sensitivity, greatly different subsets of genes sequentially participated in cellular response to radiation at their specific activation or deactivation time points. The majority of radiation-responsive genes were differentially but not commonly expressed between radiation-resistant and -sensitive cells. When these differentially regulated genes were classified according to their physiological and functional characteristics and radiation sensitivity, it was prominently obvious that DNA repair-promoting genes were up-regulated in radio-resistant cells and down-regulated or unchanged in radiation-sensitive cells. The present findings indicate that different subsets of genes are sequentially working and DNA repair capacity may control the radiation sensitivity of human hepatocellular carcinoma cells more than any other physiological factor.

Correspondence to: Dr Kee-Ho Lee, Laboratory of Molecular Oncology, Korea Institute of Radiological and Medical Sciences, 215-4 Gongneung-dong, Nowon-Ku, Seoul 139-706, Korea
E-mail: khlee@kcch.re.kr

Key words: cDNA microarray, hepatocellular carcinoma, radiation, DNA repair

Introduction

Hepatocellular carcinoma (HCC) is one of the most fatal diseases in the world (1). Although there are many treatment modalities of HCC, including surgical resection, radiotherapy, percutaneous ethanol injection therapy, liver transplantation and transcatheter arterial chemo-embolisation (2), surgical resection is potentially the only form of curative treatment (3). However, only <20% of HCC patients are surgical candidates at the time of diagnosis, either due to frequent metastasis or inadequate hepatic reserve to permit hepatectomy to be a practical treatment option (4). Radiotherapy, an alternative treatment modality for non-surgical candidate HCC patients, is not, however, more effective than other modalities because of a low yield of tumor regression, radiation-induced liver diseases, and impaired liver function due to severe liver cirrhosis (5-7). The poor response rate of HCC to radiation might also be due to an intrinsic resistance to radiotherapy, including anticancer chemotherapy. Nevertheless, amidst the wide array of non-surgical modalities available, radiotherapy has been thought to offer another interesting choice for the treatment of HCC (8).

The development of cDNA microarray technology made it possible to simultaneously analyze multiple genetic markers and subsequently categorize human cancer subtypes according to their responses to anticancer agents (9). However, despite a plethora of gene expression data of various human cancers treated with anticancer agents, including radiation (10), none were found to have examined the response of HCC against irradiation. Therefore, the present study was undertaken to investigate the temporal gene expression profile of HCC after irradiation using cDNA microarray and we identified DNA repair-associated genes that could contribute to radiation-resistant characteristics of the HCC cell line. Our present expression profile data of radiation-responsive genes are expected to provide an informed approach to therapeutic targets for enhancement of the radiation sensitivity of HCC.

Materials and methods

Cell cultures. HepG2, Hep3B, SK-Hep-1 and PLC/PRF/5 were cultured in minimal essential medium supplemented with 10% fetal bovine serum (JRH Biosciences, Lenexa, KS, USA). Huh-7, Chang liver, SNU-182 and SNU-398 cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum. The cells were maintained in a humidified incubator with 5% CO_2 at 37˚C.

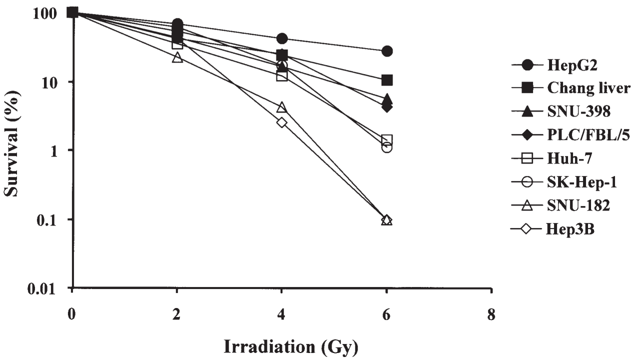

Figure 1. Survival analysis of HCC cell lines exposed to radiation. Eight different HCC cell lines were exposed to 4 Gy ionizing radiation, and the surviving colonies were counted as described in Materials and methods.

Measurement of radiation sensitivity. The radiation sensitivity of human hepatocellular carcinoma cell lines was determined by measuring the colony-forming ability of single cells exposed to radiation. Cells were seeded in triplicate in 6-cm culture dishes and exposed to γ-rays using a ^{137}Cs source, and resulting colonies formed 12 days after irradiation were stained with 0.1% crystal violet for 30 min. The relative cell survival was calculated as the percentage of colonies counted in unirradiated cohorts.

Preparation of total cellular RNA for cDNA microarray. HepG2 and Hep3B cells were seeded onto 10-cm culture dishes and harvested at 0, 3, 24 and 72 h after exposure to ionizing radiation at a dose of 4 Gy. Total cellular RNA was extracted using an RNeasy Mini kit (Qiagen, Hilden, Germany), and

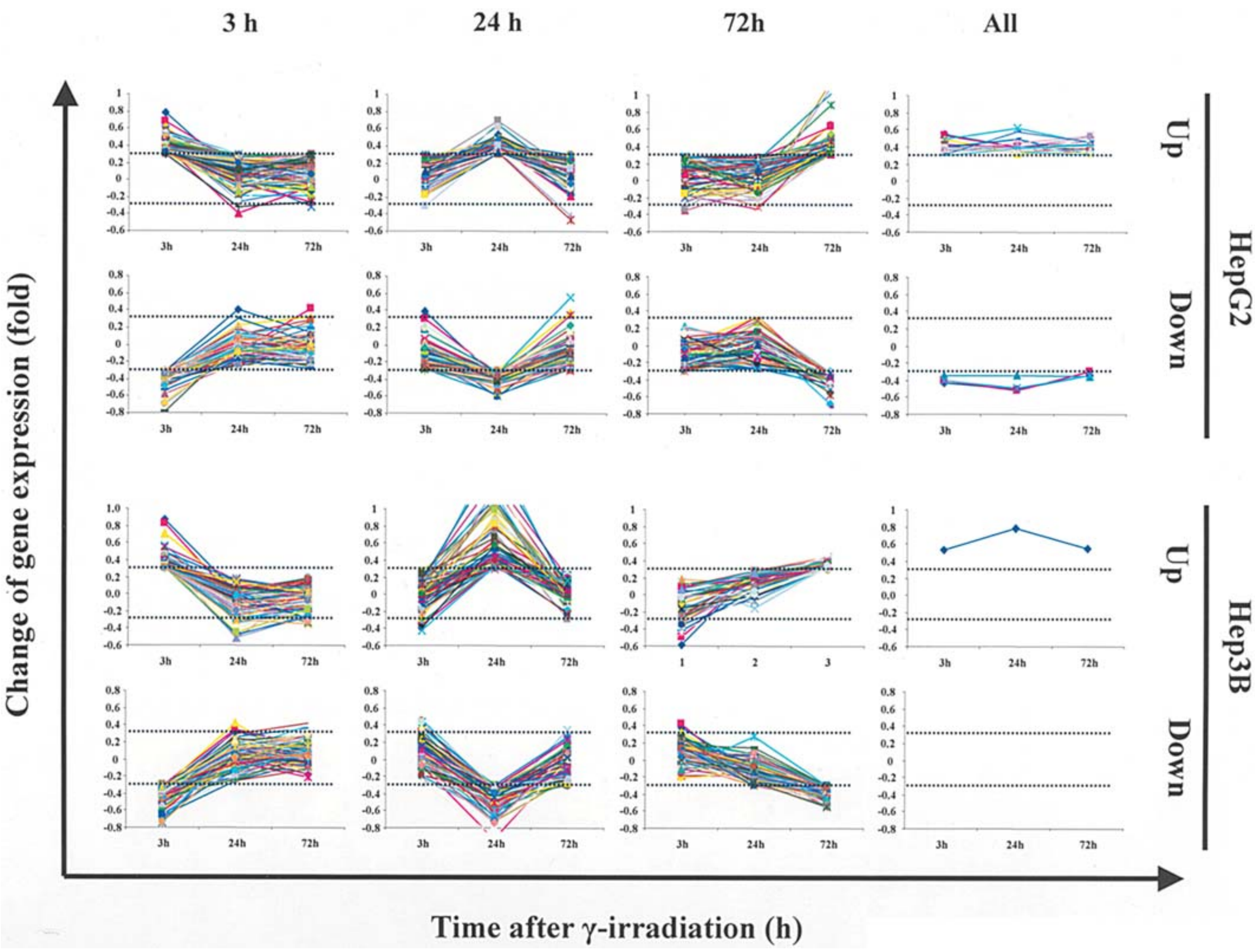

Figure 2. Temporal analysis of gene expression variation following exposure to radiation. The radiation-responsive genes with >2-fold up-regulation or <0.5-fold down-regulation were selected at 3 h (first column from left), 24 h (second column from left), and 72 h (third column from left) following 4 Gy radiation, respectively; their expression variations are plotted at the three time points indicated on the X axis. The range of expression levels within the above mentioned zone is indicated by a dashed line (------). Constitutively up- and down-regulated genes during the period of 72 h post irradiation are plotted on the right column.

ime-course distribution of number of genes showing expression variation.

A. HepG2

	3 h			24 h			72 h		
Fold	Up	Down	Total	Up	Down	Total	Up	Down	Total
1.5	257	252	509	339	341	680	295	305	600
2.0	142	79	221	111	140	251	98	112	210
3.0	33	11	44	21	19	40	20	6	26

B. Hep3B

	3 h			24 h			72 h		
Fold	Up	Down	Total	Up	Down	Total	Up	Down	Total
1.5	434	594	1028	362	280	642	339	452	791
2.0	79	187	266	99	167	266	51	251	302
3.0	9	28	37	40	59	99	0	29	29

The number of genes with altered expression were summarized at three time points of 3, 24, and 72 h after 4 Gy irradiation. Fold indicates ratios of gene expression in irradiated cells divided by those of unirradiated control cells. Up or down indicates expression ratios above or below 1.5-, 2.0-, and 3.0-fold, respectively.

isolated RNA was confirmed by gel electrophoresis and ethidium bromide staining.

cDNA microarray analysis. The 14 K cDNA microarray chips containing 14,080 genes and 704 control genes were printed onto glass microscope slides using Korean Unigene Information cDNA clones. Probe preparation and hybridization were performed using a 3DNA Array 50 kit (Genisphere Inc., PA, USA) according to the manufacturer's protocol. Briefly, 5 μg each of experimental RNA of irradiated cells and 5 μg of reference RNA obtained from unirradiated cells were reverse transcribed with Cy5/Alexa Fluor 647 and Cy3/Alexa Fluor 546 primers, respectively, by incubation with reverse transcriptase for 2 h. Two cDNAs with Cy-3 or Cy-5 capture sequences were mixed and hybridized in Express Hyb™ solution (BD Biosciences, Palo Alto, CA, USA) onto microarray overnight at 65˚C. The arrays were washed and scanned by a ScanArray scanner (Perkin-Elmer, Boston, MA, USA).

Data analysis. Primary data from image files were obtained using Imagene 5.0 (Bio-discovery, Marina del Rey, CA, USA) and normalized using an intensity and spatially-dependent method, as previously described (9). All array spot elements, for which the fluorescent intensity in each channel was >1.4-fold the local background, were considered well measured. The primary array data can be obtained at the website (http://www.kiramsdb.re.kr/db/lkh1.html).

Results

Continuous activation of a different subset of genes in response to radiation. To obtain radiation sensitivity profiles of HCC cell lines, we performed a colony forming assay after exposure of eight HCC cell lines to various doses of ionizing radiation. They were subsequently dissected according to the level of radiation sensitivity: HepG2, Chang liver, SNU-398, and PLC/PRF/5 cell lines were categorized as a relatively radiation-resistant group, and Huh-7, SK-Hep-1, SNU-182 and Hep3B cell lines as a relatively radiation-sensitive group (Fig. 1). Using this radiosensitivity profile, we selected two HCC cell lines with the most differential sensitivity among them: HepG2 and Hep3B were chosen to be representative radiation-resistant and -sensitive lines, respectively.

To identify whether genes responded differentially or commonly between radio-sensitive and -resistant HCC cell lines, we performed a cDNA microarray of HepG2 and Hep3B cells and then analyzed the temporal variation of 14,080 gene expression levels upon exposure to 4 Gy radiation. One prominent finding was the occurrence of a subset of radiation-responsive genes whose expression levels varied as a function of time in both cell lines. These radiation-responsive genes were largely divided into three groups on the basis of their expressional variation starting points: immediate response genes (Fig. 2, first column from left), intermediate response genes (Fig. 2, second column from left), and late response genes (Fig. 2, third column from left), whose expressions were up- or down-regulated within 3, 24, and 72 h, respectively. The most common characteristics of these radiation-responsive genes were that the altered expression levels of the majority of immediate and intermediate response genes were markedly recovered to their original levels at 24 and 72 h, respectively, and that late response genes did not show any significant changes in their expression levels at 3 and 24 h. Of these radiation-responsive genes in HepG2 (n=369) or Hep3B cells (n=230), however, the genes that continuously maintained altered levels constituted only 4.87% or 0.43% (Fig. 2, right column), respectively, indicating that the majority of radiation-responsive genes in each group have their specific allocated periods in which to participate in the process of cellular response to radiation. As summarized in Table I, which shows the number of genes with altered expression following radiation exposure, radiation-responsive genes with a >2-fold up-regulation or <0.5-fold down-regulation during the period

Table II. Commonly up-regulated genes between radiation-resistant and -sensitive cells following radiation exposure.

A. At 3 h.

	GenBank	Name	Symbol	HepG2	Hep3B
1	NM_002901	Reticulocalbin 1, EF-hand calcium binding domain	RCN1	3.48[a]	1.84
2	AK000405	Ubiquitin-like 4	UBL4	3.37	2.03
3	NM_003918	Glycogenin 2	GYG2	2.77	1.90
4	AF082557	Tankyrase	TNKS	2.73	1.89
5	AK027693	Myeloid-associated differentiation marker	MYADM	2.85	1.98
6	NM_007108	Transcription elongation factor B2	TCEB2	2.59	1.84
7	NM_004024	Activating transcription factor 3	ATF3	2.54	1.81
8	NM_005132	REC8-like 1 (yeast)	REC8L1	2.75	2.07
9	AF009674	Axin 1	AXIN1	3.06	2.34
10	D31763	Zinc finger protein 33a (KOX 31)	ZNF33A	2.43	1.91
11	NM_014078	Mitochondrial ribosomal protein L13	MRPL13	2.23	1.78
12	NM_002668	Proteolipid protein 2 (colonic epithelium-enriched)	PLP2	2.96	2.44
13	NM_017425	Sperm autoantigenic protein 17	SPA17	2.39	2.01
14	NM_005749	Transducer of ERBB2, 1	TOB1	2.27	2.45
15	NM_002836	Protein tyrosine phosphatase, receptor type, A	PTPRA	2.38	2.57
16	AW572940	Signal-induced proliferation-associated 1 like 3	SIPA1L3	2.02	2.46
17	NM_017793	RNase P protein subunit p25	Rpp25	2.10	2.63
18	NM_018927	Protocadherin γ subfamily C, 3	PCDHGC3	2.12	2.79

B. At 24 h.

	GenBank	Name	Symbol	HepG2	Hep3B
1	BG289963	Membrane targeting (tandem) C2 domain containing 1	MTAC2D1	3.68[a]	2.03
2	AF038196	Radical fringe homolog (*Drosophila*)	RFNG	3.09	1.99
3	BI334171	Filamin-binding LIM protein-1	FBLP-1	3.22	2.21
4	NM_006838	Methionyl aminopeptidase 2	METAP2	2.58	1.77
5	NM_003576	Serine/threonine kinase 24	STK24	2.37	1.78
6	NM_004363	CEACAM5	CEACAM5	2.46	1.89
7	NM_000308	Protective protein for ß-galactosidase	PPGB	2.89	2.27
8	NM_012429	SEC14-like 2 (*S. cerevisiae*)	SEC14L2	2.49	2.03
9	BC005066	SMARCE1	SMARCE1	2.17	1.81
10	BC009367	Zinc finger protein 317	ZNF317	2.47	2.11
11	NM_003600	Serine/threonine kinase 6	STK6	2.10	1.81
12	NM_002610	Pyruvate dehydrogenase kinase, isoenzyme 1	PDK1	2.07	1.83
13	NM_001067	Topoisomerase (DNA) II α, 170 kDa	TOP2A	2.14	1.94
14	AK023129	HP1-BP74	HP1-BP74	2.07	1.88
15	NM_016237	Anaphase promoting complex subunit 5	ANAPC5	1.97	1.80
16	NM_005643	TAF11 RNA polymerase II, 28 kDa	TAF11	1.96	1.80
17	BC014253	Golgi membrane protein SB140	SMAP-5	2.02	1.89
18	NM_006468	Polymerase (RNA) III (DNA directed), 62 kDa	RPC62	1.97	1.91
19	NM_007210	GALNT6	GALNT6	1.97	1.96
20	AL049998	Phosphoinositide-3-kinase, class 2, α polypeptide	PIK3C2A	2.19	2.35
21	N23258	Family with sequence similarity 31, member C	FAM31C	2.03	2.20
22	NM_006666	RuvB-like 2 (*E. coli*)	RUVBL2	1.95	2.15
23	BC010629	Outer dense fiber of sperm tails 2	ODF2	2.46	2.77
24	NM_005999	Translin-associated factor X	TSNAX	2.66	5.79
25	NM_002856	Poliovirus receptor-related 2	PVRL2	0.83	2.04
26	NM_004508	Isopentenyl-diphosphate δ isomerase	IDI1	2.14	6.61

C. At 72 h.

	GenBank	Name	Symbol	HepG2	Hep3B
1	NM_003244	TGFB-induced factor (TALE family homeobox)	TGIF	3.09[a]	1.78
2	NM_006899	Isocitrate dehydrogenase 3 (NAD$^+$) ß	IDH3B	3.41	2.15

Continued.

	GenBank	Name	Symbol	HepG2	Hep3B
3	BG354575	Cell division cycle associated 2	CDCA2	2.47	1.79
4	NM_022170	Williams-Beuren syndrome chromosome region 1	WBSCR1	2.33	1.75
5	AF163473	Protein phosphatase 2A, ß isoform	PPP2R1B	2.47	1.93
6	BG325046	TNF receptor-associated factor 3	TRAF3	2.13	1.72
7	NM_001096	ATP citrate lyase	ACLY	2.09	1.72
8	NM_001865	Cytochrome C oxidase subunit VIIa polypeptide 2	COX7A2	2.17	1.79
9	NM_012429	SEC14-like 2 (*S. cerevisiae*)	SEC14L2	2.17	1.80
10	BE253334	Cytochrome C oxidase subunit VIa polypeptide 1	COX6A1	2.08	1.74
11	NM_003503	CDC7 cell division cycle 7 (*S. cerevisiae*)	CDC7	2.03	1.75
12	NM_001443	Fatty acid binding protein 1, liver	FABP1	2.33	2.04
13	Z17227	Interleukin 10 receptor, ß	IL10RB	2.33	2.06
14	NM_003366	Ubiquinol-cytochrome C reductase core protein II	UQCRC2	2.37	2.26
15	AK000405	Ubiquitin-like 4	UBL4	2.09	2.27
16	NM_013238	DnaJ (Hsp40) homolog, subfamily D, member 1	DNAJD1	1.99	2.21
17	NM_003127	Spectrin, alpha, non-erythrocytic 1 (α-fodrin)	SPTAN1	1.91	2.17
18	NM_006328	RNA binding motif protein 14	RBM14	2.00	2.35

From the top 200 genes up-regulated at 3, 24, and 72 h in HepG2 or Hep3B cells after 4 Gy irradiation, overlapped genes between them are listed in A, B, and C, respectively. Cut-off ratios of Cy5/Cy3 of the top 200 up-regulated genes in HepG2 and Hep3B cells were 2.00 and 1.74 at 3 h, 1.92 and 1.77 at 24 h, and 1.89 and 1.71 at 72 h, respectively. [a]Intensity ratios of Cy5/Cy3.

of 72 h were 682 (12.7%) and 834 (15.5%) in HepG2 and Hep3B cells, respectively, out of a total 5,364 informative genes. Furthermore, a similar number of radiation-responsive genes at each time point of 3, 24, or 72 h post-irradiation was sequentially working during the period of 72 h (221 vs. 251 vs. 210 in HepG2 cells, and 266 vs. 266 vs. 302 in Hep3B cells, respectively). The above findings, which were analyzed on the basis of temporal expression of radiation-responsive genes, indicate that remarkably different subsets of genes were sequentially up- or down-regulated at their activation or deactivation time points upon exposure to radiation, and >13% of genes actively participated in the radiation-induced DNA damage response, irrespective of HCC cell radiation sensitivity.

HCC radiation sensitivity primarily dependent on DNA repair gene family. To identify a more reliable subset of radiation-responsive genes, we then examined commonly up- or down-regulated genes in both HepG2 and Hep3B cells following radiation exposure using the above-observed expression profiles. Of the 200 most highly up-regulated genes in each cell line, overlapped genes at 3, 24, and 72 h numbered only 18, 26, and 18, respectively (Table II). Similar to up-regulated genes, a low number of overlapped genes was also found among the bottom 200 down-regulated genes in each cell line (Table III; 22, 57, and 17 genes at 3, 24, and 72 h, respectively), indicating that the number of commonly responsive genes was few among radiation-responsive genes in each HCC cell line.

The small number of overlapped genes may have been due to a large difference of radiation sensitivity between HepG2 and Hep3B cell lines. This notion led us to consider the possibility that the determination of radiation sensitivity might be associated with genes expressed differentially rather than commonly between HepG2 and Hep3B cells (Tables IV and V). To identify a group of genes that are functionally and physiologically responsible for radiation sensitivity, we classified radiation-responsive genes by running the FatiGO gene ontology algorithm (http://fatigo.bioinfo.cnio.es) (11) and then identified differentially expressed genes within a specific expression cluster between HepG2 and Hep3B cells. We initially focused on DNA repair-associated genes, since DNA repair is an important mechanistic basis of healing single- and double-stranded breaks upon radiation exposure. In an effort to classify DNA repair-associated genes according to their actions, we separated them into four categories based on repair mechanisms: base excision repair (BER), nucleotide excision repair (NER), double-strand break repair (DSB), and mismatch repair (MMR) (Table VI). Strikingly, it was found that DNA repair-promoting genes were up-regulated in HepG2 cells, while their expression levels were unchanged or down-regulated in Hep3B cells. Of genes operating on mismatch repair, MSH6 (12) in HepG2 cells was up-regulated at 3 h, and PMS1 (13) in Hep3B cells was down-regulated at 24 h. In addition, HepG2 cells showed an up-regulation of genes involved in double-strand break repair, such as Ku70 and DNA-activated protein kinase, and nucleotide excision repair, such as ERCC5. In contrast to the increase of DNA repair gene expression levels specifically in radio-resistant HepG2 cells, radio-sensitive Hep3B cells showed a down-regulation of base excision repair genes, such as uracil-DNA glycosylase-1 and -2 genes, in addition to Ku70, ERCC5, and PMS1. Since a correlation between radiosensitivity and expression variation of DNA repair gene family was not found under any other physiological conditions, such as apoptosis and cell growth (data not shown), the present findings led us to conclude that the DNA repair gene family is responsible for the differential radiosensitivity in HCC.

Table III. Commonly down-regulated genes between radiation-resistant and -sensitive cells following radiation exposure.

A. At 3 h.

	GenBank	Name	Symbol	HepG2	Hep3B
1	NM_007145	Zinc finger protein 146	ZNF146	0.55[a]	0.18
2	NM_020640	RP42 homolog	RP42	0.54	0.24
3	AB051509	Glucocorticoid receptor DNA binding factor 1	GRLF1	0.53	0.27
4	NM_007355	Heat shock 90 kDa protein 1, ß	HSPCB	0.42	0.23
5	NM_001418	Eukaryotic translation initiation factor 4 γ, 2	EIF4G2	0.51	0.30
6	NM_003392	Wingless-type MMTV integration site family, member 5A	WNT5A	0.51	0.33
7	NM_003295	Tumor protein, translationally-controlled 1	TPT1	0.54	0.36
8	NM_005165	Aldolase C, fructose-bisphosphate	ALDOC	0.54	0.37
9	NM_012425	Ras suppressor protein 1	RSU1	0.53	0.40
10	NM_005626	Splicing factor, arginine/serine-rich 4	SFRS4	0.55	0.45
11	NM_001614	Actin, γ 1	ACTG1	0.55	0.45
12	NM_002531	Neurotensin receptor 1 (high affinity)	NTSR1	0.49	0.42
13	NM_006869	Centaurin, α 1	CENTA1	0.52	0.45
14	NM_003406	YWHAZ	YWHAZ	0.54	0.49
15	NM_018950	Major histocompatibility complex, class I, F	HLA-F	0.48	0.43
16	NM_018929	Protocadherin γ subfamily C, 3	PCDHGC3	0.55	0.50
17	AK026813	Six transmembrane epithelial antigen of prostate 2	STEAP2	0.44	0.41
18	NM_012242	Dickkopf homolog 1 (*Xenopus laevis*)	DKK1	0.50	0.47
19	NM_005776	Cornichon homolog (*Drosophila*)	CNIH	0.49	0.47
20	NM_014166	Vitamin D receptor interacting protein	VDRIP	0.33	0.33
21	NM_015846	Methyl-CpG binding domain protein 1	MBD1	0.40	0.43
22	NM_003073	SMARCB1	SMARCB1	0.29	0.50

B. At 24 h.

	GenBank	Name	Symbol	HepG2	Hep3B
1	BG742113	Small proline rich-like (epidermal differentiation complex) 2A	SPRL2A	0.53[a]	0.16
2	NM_002950	Ribophorin I	RPN1	0.52	0.29
3	NM_004957	Folylpolyglutamate synthase	FPGS	0.50	0.31
4	AK025949	Poliovirus receptor	PVR	0.41	0.26
5	NM_006387	Calcium homeostasis endoplasmic reticulum protein	CHERP	0.46	0.31
6	AV751729	T84 colon carcinoma cell IL-1ß-regulated HSCC1 mRNA		0.47	0.31
7	AL541868	Layilin	LOC143903	0.52	0.35
8	NM_024647	Nucleoporin, 43 kDa	NUP43	0.46	0.33
9	NM_015976	Sorting nexin 7	SNX7	0.48	0.35
10	BC014396	Copine IV	CPNE4	0.45	0.33
11	BG340148	HESB like domain containing 1	HBLD1	0.37	0.29
12	AL157424	Synaptojanin 2	SYNJ2	0.49	0.39
13	NM_006947	Signal recognition particle, 72 kDa	SRP72	0.48	0.38
14	NM_006442	DR1-associated protein 1 (negative cofactor 2 α)	DRAP1	0.52	0.41
15	NM_014214	Inositol(myo)-1(or 4)-monophosphatase 2	IMPA2	0.47	0.37
16	NM_005178	B-cell CLL/lymphoma 3	BCL3	0.52	0.42
17	NM_032905	RNA binding motif protein 17	RBM17	0.31	0.26
18	NM_001439	Exostoses (multiple)-like 2	EXTL2	0.39	0.32
19	NM_024513	FYVE and coiled-coil domain containing 1	FYCO1	0.50	0.41
20	NM_007283	Monoglyceride lipase	MGLL	0.49	0.41
21	NM_032871	Tumor necrosis factor receptor superfamily, member 19-like	TNFRSF19L	0.52	0.44
22	M83822	LPS-responsive vesicle trafficking, beach and anchor containing	LRBA	0.41	0.35
23	NM_006762	Lysosomal-associated multispanning membrane protein-5	LAPTM5	0.49	0.42
24	BC001092	Congenital dyserythropoietic anemia, type I	CDAN1	0.48	0.41
25	AL117459	RAD51-like 3 (*S. cerevisiae*)	RAD51L3	0.42	0.36
26	NM_004929	Calbindin 1, 28 kDa	CALB1	0.44	0.39
27	AK025781	Sorting nexin 12	SNX12	0.42	0.38

Continued.

	GenBank	Name	Symbol	HepG2	Hep3B
28	NM_024017	Homeobox B9	HOXB9	0.47	0.42
29	NM_030763	Nucleosomal binding protein 1	NSBP1	0.43	0.39
30	NM_016652	Crn, crooked neck-like 1 (*Drosophila*)	CRNKL1	0.46	0.42
31	AF208850	Protein tyrosine phosphatase type IVA, member 2	PTP4A2	0.31	0.28
32	NM_004616	Transmembrane 4 superfamily member 3	TM4SF3	0.43	0.40
33	NM_004584	RAD9 homolog A (*S. pombe*)	RAD9A	0.40	0.37
34	NM_000259	Myosin VA (heavy polypeptide 12, myoxin)	MYO5A	0.37	0.34
35	BG749845	Metallothionein 2A	MT2A	0.41	0.39
36	NM_001482	Glycine amidinotransferase (L-arginine:glycine amidinotransferase)	GATM	0.45	0.44
37	M18216	CEACAM6	CEACAM6	0.45	0.45
38	AV726409	Ariadne homolog 2 (*Drosophila*)	ARIH2	0.40	0.40
39	NM_002933	Ribonuclease, RNase A family, 1 (pancreatic)	RNASE1	0.44	0.44
40	NM_033027	AXIN1 up-regulated 1	AXUD1	0.41	0.42
41	NM_024532	PF20	PF20	0.41	0.43
42	BI488702	Metallothionein 1K	MT1K	0.43	0.45
43	BF115152	G protein-coupled receptor 110	GPR110	0.24	0.25
44	NM_004375	COX11 homolog, cytochrome C oxidase assembly protein (yeast)	COX11	0.34	0.38
45	BC013603	Zinc finger protein 3 (A8-51)	ZNF3	0.26	0.29
46	NM_000149	Fucosyltransferase 3	FUT3	0.35	0.39
47	NM_015550	Oxysterol binding protein-like 3	OSBPL3	0.33	0.37
48	AL353934	MUS81 endonuclease homolog (yeast)	MUS81	0.36	0.41
49	NM_002673	Plexin B1	PLXNB1	0.28	0.32
50	NM_004178	TAR (HIV) RNA binding protein 2	TARBP2	0.39	0.45
51	AA400519	Polyhomeotic like 3 (*Drosophila*)	PHC3	0.29	0.34
52	NM_006405	Transmembrane 9 superfamily member 1	TM9SF1	0.28	0.34
53	BE888791	Hepatocellular carcinoma-related protein 1	FLJ32642	0.30	0.37
54	NM_006458	Tripartite motif-containing 3	TRIM3	0.35	0.44
55	BC001043	Splicing factor 4	SF4	0.28	0.38
56	NM_005652	Telomeric repeat binding factor 2	TERF2	0.25	0.38
57	NM_005186	Calpain 1, (mu/I) large subunit	CAPN1	0.19	0.39

C. At 72 h.

	GenBank	Name	Symbol	HepG2	Hep3B
1	AA400519	Polyhomeotic like 3 (*Drosophila*)	PHC3	0.53[a]	0.26
2	NM_002673	Plexin B1	PLXNB1	0.51	0.27
3	NM_006405	Transmembrane 9 superfamily member 1	TM9SF1	0.52	0.28
4	BC013603	Zinc finger protein 3 (A8-51)	ZNF3	0.51	0.28
5	NM_012116	Cas-Br-M (murine) ecotropic retroviral transforming sequence C	CBLC	0.52	0.29
6	BE888791	Hepatocellular carcinoma related protein 1	FLJ32642	0.49	0.31
7	NM_015550	Oxysterol binding protein-like 3	OSBPL3	0.46	0.30
8	NM_000149	Fucosyltransferase 3	FUT3	0.51	0.36
9	BG742113	Small proline rich-like (epidermal differentiation complex) 2A	SPRL2A	0.46	0.34
10	NM_014595	5', 3'-nucleotidase, cytosolic	NT5C	0.45	0.33
11	NM_016733	LIM domain kinase 2	LIMK2	0.50	0.38
12	NM_003186	Transgelin	TAGLN	0.45	0.34
13	NM_006317	Brain abundant, membrane attached signal protein 1	BASP1	0.52	0.40
14	NM_018282	Paraspeckle component 1	PSPC1	0.51	0.40
15	NM_030763	Nucleosomal binding protein 1	NSBP1	0.52	0.41
16	NM_024532	PF20	PF20	0.53	0.44
17	NM_006680	Malic enzyme 3, NADP(+)-dependent, mitochondrial	ME3	0.46	0.40

From the bottom 200 genes down-regulated in HepG2 or Hep3B cells, overlapped genes between them are listed at their respective time points, 3 h (A), 24 h (B), and 72 h (C) after 4 Gy irradiation. Cut-off ratios of Cy5/Cy3 of the bottom 200 genes down-regulated in HepG2 and Hep3B cells were 0.56 and 0.51 at 3 h, 0.53 and 0.45 at 24 h, and 0.55 and 0.44 at 72 h, respectively. [a]Intensity ratios of Cy5/Cy3.

Table IV. Differentially up-regulated genes between radiation-resistant and -sensitive cells following radiation exposure.

A. At 3 h.

	GenBank	Name	Symbol	Rank	HepG2	Hep3B
1	NM_022371	Torsin family 3, member A	TOR3A	5.31	2.57[a]	0.48
2	NM_004083	Methionine-tRNA synthetase	MARS	5.29	3.04	0.57
3	BI757349	Ectonucleoside triphosphate diphosphohydrolase 7	ENTPD7	4.68	2.61	0.56
4	AL137548	Vitamin K epoxide reductase complex, subunit 1-like 1	VKORC1L1	4.62	3.18	0.69
5	NM_004339	Pituitary tumor-transforming 1 interacting protein	PTTG1IP	4.55	3.67	0.81
6	BG739896	Tetratricopeptide repeat domain 14	TTC14	4.48	4.72	1.05
7	NM_005517	High-mobility group nucleosomal binding domain 2	HMGN2	4.39	3.07	0.70
8	NM_025151	Rab coupling protein	RCP	4.20	3.68	0.88
9	NM_012260	2-hydroxyphytanoyl-CoA lyase	HPCL2	4.12	3.33	0.81
10	NM_000919	Peptidylglycine alpha-amidating monooxygenase	PAM	3.96	2.76	0.70
11	NM_000179	MutS homolog 6 (*E. coli*)	MSH6	3.76	3.67	0.98
12	AA011320	Spectrin, ß, erythrocytic	SPTB	3.74	2.77	0.74
13	NM_004521	Kinesin family member 5B	KIF5B	3.73	3.12	0.83
14	NM_021983	Major histocompatibility complex, class II, DR ß 3	HLA-DRB3	3.68	2.12	0.58
15	BC009367	Zinc finger protein 317	ZNF317	3.65	3.46	0.95
16	NM_003897	Immediate early response 3	IER3	3.62	3.10	0.86
17	NM_006784	WD repeat domain 3	WDR3	3.60	6.05	1.68
18	NM_020189	DC6 protein	DC6	3.58	2.79	0.78
19	BI667192	Histidyl-tRNA synthetase 2	HARS2	3.58	3.71	1.04
20	NM_005420	Sulfotransferase family 1E, estrogen-preferring 1	SULT1E1	3.49	2.50	0.72
21	NM_003143	Single-stranded DNA binding protein 1	SSBP1	3.47	2.61	0.75
22	NM_005038	Peptidylprolyl isomerase D (cyclophilin D)	PPID	3.45	3.94	1.14
23	NM_017595	I-κB-interacting Ras-like protein 2	KBRAS2	3.40	2.24	0.66
24	NM_022145	Leucine zipper protein FKSG14	FKSG14	3.37	3.85	1.14
25	NM_003758	Eukaryotic translation initiation factor 3, subunit 1 α	EIF3S1	3.34	2.43	0.73
26	NM_004729	Zinc finger, BED domain containing 1	ZBED1	3.30	3.23	0.98
27	AL136877	SMC4 structural maintenance of chromosomes 4-like 1	SMC4L1	3.29	2.10	0.64
28	H08440	Ret finger protein	RFP	3.25	3.99	1.23
29	BC000195	DORA reverse strand protein 1	DREV1	3.23	2.43	0.75
30	AL136833	mSin3A-associated protein 130	SAP130	3.19	3.49	1.09
31	NM_001251	CD68 antigen	CD68	3.11	2.29	0.74
32	NM_006711	RNA binding protein S1, serine-rich domain	RNPS1	3.01	2.95	0.98
33	NM_020202	Nit protein 2	NIT2	3.01	2.36	0.79
34	AB023195	Additional sex combs like 1 (*Drosophila*)	ASXL1	0.33	0.52	1.57
35	AB020711	CDC2-related protein kinase 7	CRK7	0.33	0.41	1.25
36	NM_014970	Kinesin-associated protein 3	KIFAP3	0.33	0.54	1.63
37	AL080157	WD repeat domain 21	WDR21	0.33	0.52	1.58
38	BG427872	Ubiquitin specific protease 46	USP46	0.33	0.52	1.57
39	AF208850	Protein tyrosine phosphatase type IVA, member 2	PTP4A2	0.33	0.47	1.44
40	AL512725	Midnolin	MIDN	0.33	0.43	1.32
41	BE888791	Hepatocellular carcinoma related protein 1	FLJ32642	0.33	0.39	1.20
42	AA400519	Polyhomeotic like 3 (*Drosophila*)	PHC3	0.32	0.37	1.14
43	NM_021170	bHLH factor Hes4	Hes4	0.32	0.45	1.41
44	NM_014501	Ubiquitin-conjugating enzyme E2S	UBE2S	0.32	0.40	1.27
45	NM_014595	5', 3'-nucleotidase, cytosolic	NT5C	0.31	0.37	1.17
46	D30612	Zinc finger protein 282	ZNF282	0.31	0.49	1.55
47	BC001043	Splicing factor 4	SF4	0.31	0.42	1.36
48	NM_018688	Bridging integrator 3	BIN3	0.31	0.41	1.35
49	NM_001439	Exostoses (multiple)-like 2	EXTL2	0.30	0.46	1.53
50	BG340148	HESB like domain containing 1	HBLD1	0.30	0.45	1.52

Continued.

	GenBank	Name	Symbol	Rank	HepG2	Hep3B
51	NM_005186	Calpain 1, (mu/I) large subunit	CAPN1	0.30	0.38	1.30
52	NM_003739	Aldo-keto reductase family 1, member C3	AKR1C3	0.29	0.47	1.60
53	NM_002673	Plexin B1	PLXNB1	0.29	0.44	1.53
54	NM_007246	Kelch-like 2, Mayven (*Drosophila*)	KLHL2	0.29	0.50	1.73
55	NM_002226	Jagged 2	JAG2	0.28	0.46	1.61
56	NM_002661	Phospholipase C, γ 2	PLCG2	0.28	0.48	1.72
57	NM_012116	Cas-Br-M ecotropic retroviral transforming sequence C	CBLC	0.27	0.45	1.63
58	NM_001703	Brain-specific angiogenesis inhibitor 2	BAI2	0.27	0.50	1.85
59	BF115152	G protein-coupled receptor 110	GPR110	0.27	0.37	1.36
60	NM_000819	Phosphoribosylglycinamide formyltransferase	GART	0.27	0.54	2.02
61	AF207881	Cytosolic ovarian carcinoma antigen 1	COVA1	0.26	0.55	2.16
62	NM_012164	F-box and WD-40 domain protein 2	FBXW2	0.24	0.17	0.68
63	NM_015953	Nitric oxide synthase interacting protein	NOSIP	0.24	0.43	1.80
64	BG254520	MADS box transcription enhancer factor 2A	MEF2A	0.23	0.33	1.44
65	NM_005652	Telomeric repeat binding factor 2	TERF2	0.22	0.39	1.79
66	AF277181	PNAS-123	LOC85028	0.22	0.37	1.71
67	NM_003686	Exonuclease 1	EXO1	0.19	0.20	1.05
68	NM_005873	Regulator of G-protein signalling 19	RGS19	0.19	0.34	1.80
69	D87452	Inositol hexaphosphate kinase 1	IHPK1	0.18	0.48	2.72
70	NM_016434	Tumor necrosis factor receptor superfamily, member 6b	TNFRSF6B	0.15	0.54	3.64

B. At 24 h.

	GenBank	Name	Symbol	Rank	HepG2	Hep3B
1	NM_014426	Sorting nexin 5	SNX5	11.70	2.39[a]	0.20
2	NM_006302	Glucosidase I	GCS1	9.17	3.26	0.36
3	NM_000521	Hexosaminidase B	HEXB	7.98	2.18	0.27
4	AF080255	Transcription termination factor II	TTF2	4.45	2.27	0.51
5	AA011320	Spectrin, ß, erythrocytic	SPTB	4.42	2.98	0.67
6	NM_001966	EHHADH	EHHADH	4.41	3.38	0.77
7	NM_001281	Cytoskeleton associated protein 1	CKAP1	4.41	3.13	0.71
8	NM_000389	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	CDKN1A	4.33	2.10	0.48
9	NM_000629	Interferon (α, ß and ω) receptor 1	IFNAR1	4.21	4.85	1.15
10	NM_006899	Isocitrate dehydrogenase 3 (NAD+) ß	IDH3B	4.07	2.79	0.69
11	NM_005815	Zinc finger protein 443	ZNF443	4.05	2.08	0.51
12	NM_022333	TIA1-like 1	TIAL1	4.00	2.11	0.53
13	AK027071	Transforming growth factor ß-stimulated protein TSC-22	TSC22	3.96	3.26	0.82
14	NM_017425	Sperm autoantigenic protein 17	SPA17	3.61	2.27	0.63
15	NM_002633	Phosphoglucomutase 1	PGM1	3.55	2.52	0.71
16	NM_003244	TGFB-induced factor (TALE family homeobox)	TGIF	3.42	2.26	0.66
17	D30612	Zinc finger protein 282	ZNF282	3.40	2.52	0.74
18	AF009674	Axin 1	AXIN1	3.36	4.20	1.25
19	NM_006704	SGT1, suppressor of G2 allele of SKP1 (*S. cerevisiae*)	SUGT1	3.22	2.46	0.76
20	AL080209	cAMP responsive element binding protein 3-like 2	CREB3L2	3.19	3.42	1.07
21	NM_016397	TH1-like (*Drosophila*)	TH1L	3.13	3.39	1.08
22	AL117407	MAP3K7IP2	MAP3K7IP2	3.08	2.36	0.77
23	NM_000745	Cholinergic receptor, nicotinic, α polypeptide 5	CHRNA5	3.06	2.50	0.82
24	BF698884	DC2 protein	DC2	3.04	3.08	1.01
25	NM_012288	Translocation associated membrane protein 2	TRAM2	3.03	2.49	0.82
26	NM_007273	Repressor of estrogen receptor activity	REA	3.01	3.02	1.00
27	NM_006084	Interferon-stimulated transcription factor 3, γ	ISGF3G	3.01	2.16	0.72
28	BC002971	Chaperonin containing TCP1, subunit 5 (ε)	CCT5	0.33	0.39	1.20

Table IV. Continued.

B. At 24 h.

	GenBank	Name	Symbol	Rank	HepG2	Hep3B
29	NM_002999	Syndecan 4 (amphiglycan, ryudocan)	SDC4	0.29	0.42	1.45
30	NM_020167	Neuromedin U receptor 2	NMU2R	0.29	0.47	1.64
31	NM_005471	Glucosamine-6-phosphate deaminase 1	GNPDA1	0.26	0.24	0.94
32	NM_002276	Keratin 19	KRT19	0.24	0.45	1.84
33	NM_005143	Haptoglobin	HP	0.24	0.52	2.16
34	NM_032593	Histidine triad nucleotide binding protein 2	HINT2	0.15	0.47	3.15
35	BC012778	Calmodulin regulated spectrin-associated protein 1	CAMSAP1	0.04	0.49	13.15

C. At 72 h.

	GenBank	Name	Symbol	Rank	HepG2	Hep3B
1	NM_006709	HLA-B associated transcript 8	BAT8	27.52	14.10[a]	0.51
2	AF124145	Autocrine motility factor receptor	AMFR	21.08	13.76	0.65
3	NM_002868	RAB5B, member RAS oncogene family	RAB5B	10.74	12.20	1.14
4	NM_001560	Interleukin 13 receptor, α 1	IL13RA1	6.92	10.32	1.49
5	BC010464	Mitogen-activated protein kinase kinase kinase 3	MAP3K3	5.17	2.09	0.40
6	NM_002131	High mobility group AT-hook 1	HMGA1	5.09	7.70	1.51
7	AF204231	Golgin-67	GOLGIN67	4.63	4.29	0.93
8	NM_020167	Neuromedin U receptor 2	NMU2R	3.76	3.56	0.95
9	NM_002336	Low density lipoprotein receptor-related protein 6	LRP6	3.73	1.91	0.51
10	BF967508	ARP8 actin-related protein 8 homolog (yeast)	ACTR8	3.52	4.47	1.27
11	NM_005980	S100 calcium binding protein P	S100P	3.51	2.05	0.59
12	NM_005716	Regulator of G-protein signalling 19 interacting protein 1	RGS19IP1	3.37	3.33	0.99
13	AK024863	Dynein, cytoplasmic, light intermediate polypeptide 2	DNCLI2	3.34	2.59	0.78
14	NM_013400	Replication initiator 1	REPIN1	3.10	2.01	0.65
15	NM_013989	Deiodinase, iodothyronine, type II	DIO2	3.09	2.04	0.66
16	NM_006451	Poly(A) binding protein interacting protein 1	PAIP1	3.05	3.17	1.04
17	NM_005180	COMM domain containing 3	COMMD3	0.33	0.48	1.46
18	NM_016000	tRNA nucleotidyl transferase, CCA-adding, 1	TRNT1	0.33	0.50	1.52
19	NM_005113	Golgi autoantigen, golgin subfamily a, 5	GOLGA5	0.33	0.54	1.63
20	NM_005826	Heterogeneous nuclear ribonucleoprotein R	HNRPR	0.33	0.43	1.30
21	NM_002940	ATP-binding cassette, sub-family E 1	ABCE1	0.33	0.48	1.46
22	NM_005880	DnaJ (Hsp40) homolog, subfamily A, member 2	DNAJA2	0.33	0.49	1.51
23	AB032967	Zinc finger protein 473	ZNF473	0.33	0.45	1.38
24	AK001555	RAB18, member RAS oncogene family	RAB18	0.33	0.51	1.57
25	L39061	TATA box binding protein (TBP)-associated factor	TAF1B	0.32	0.26	0.82
26	NM_003664	Adaptor-related protein complex 3, ß 1 subunit	AP3B1	0.32	0.43	1.33
27	NM_006756	Transcription elongation factor A (SII), 1	TCEA1	0.32	0.41	1.29
28	BI094140	Leucine-rich PPR-motif containing	LRPPRC	0.32	0.54	1.71
29	NM_003076	SWI/SNF related, subfamily d, member 1	SMARCD1	0.31	0.44	1.39
30	NM_032704	Tubulin α 6	TUBA6	0.31	0.33	1.08
31	BC000143	Engulfment and cell motility 2	ELMO2	0.31	0.50	1.61
32	AL545781	Mitochondrial ribosomal protein L10	MRPL10	0.30	0.31	1.02
33	NM_001316	CSE1 chromosome segregation 1-like (yeast)	CSE1L	0.30	0.41	1.34
34	NM_004060	Cyclin G1	CCNG1	0.30	0.54	1.80
35	NM_003715	Vesicle docking protein p115	VDP	0.29	0.38	1.32
36	NM_004539	Asparaginyl-tRNA synthetase	NARS	0.29	0.34	1.17
37	NM_003589	Cullin 4A	CUL4A	0.29	0.44	1.54
38	X68560	Sp3 transcription factor	SP3	0.27	0.46	1.72
39	NM_003318	TTK protein kinase	TTK	0.27	0.51	1.90
40	NM_003299	Tumor rejection antigen (gp96) 1	TRA1	0.26	0.37	1.39

Continued.

	GenBank	Name	Symbol	Rank	HepG2	Hep3B
41	NM_014750	Discs, large homolog 7 (*Drosophila*)	DLG7	0.26	0.41	1.56
42	NM_012484	Hyaluronan-mediated motility receptor (RHAMM)	HMMR	0.26	0.40	1.52
43	AB032945	Myosin VB	MYO5B	0.26	0.49	1.87
44	NM_005030	Polo-like kinase 1 (*Drosophila*)	PLK1	0.25	0.38	1.50
45	NM_007202	A kinase (PRKA) anchor protein 10	AKAP10	0.25	0.47	1.85
46	NM_005733	Kinesin family member 20A	KIF20A	0.25	0.53	2.14
47	NM_021249	Sorting nexin 6	SNX6	0.24	0.35	1.45
48	NM_031217	Kinesin family member 18A	DKFZP434G2226	0.24	0.48	2.02
49	NM_006392	Nucleolar protein 5A (56 kDa with KKE/D repeat)	NOL5A	0.23	0.21	0.91
50	NM_003136	Signal recognition particle, 54 kDa	SRP54	0.23	0.42	1.84
51	NM_003905	Amyloid ß precursor protein binding protein 1	APPBP1	0.23	0.50	2.22
52	NM_018492	T-LAK cell-originated protein kinase	TOPK	0.22	0.41	1.82
53	NM_004766	Coatomer protein complex, subunit ß 2	COPB2	0.22	0.44	2.00
54	NM_003392	Wingless-type MMTV integration site family 5A	WNT5A	0.22	0.19	0.88
55	NM_007355	Heat shock 90-kDa protein 1, ß	HSPCB	0.19	0.22	1.12
56	NM_016343	Centromere protein F, 350/400ka (mitosin)	CENPF	0.19	0.43	2.30
57	NM_005701	RNA, U transporter 1	RNUT1	0.18	0.28	1.56
58	NM_001067	Topoisomerase (DNA) II α, 170 kDa	TOP2A	0.13	0.34	2.63

From the top 200 genes up-regulated in HepG2 or Hep3B cells, as presented in the legend of Table II, we selected differentially expressed genes and then ranked their orders by dividing the expression ratios of the radiation-responsive genes of HepG2 cells by those of Hep3B cells. The rank >3 or <0.3 indicates higher or lower levels of gene expression in HepG2 cells compared to those of Hep3B cells, respectively. [a]Intensity ratios of Cy5/Cy3.

Table V. Differentially down-regulated genes between radiation-resistant and -sensitive cells following radiation exposure.

A. At 3 h.

	GenBank	Name	Symbol	Rank	HepG2	Hep3B
1	NM_022371	Torsin family 3, member A	TOR3A	5.31	2.57[a]	0.48
2	NM_000287	Peroxisomal biogenesis factor 6	PEX6	4.47	1.01	0.23
3	NM_021038	Muscleblind-like (*Drosophila*)	MBNL1	4.05	1.50	0.37
4	NM_002129	High-mobility group box 2	HMGB2	3.98	1.51	0.38
5	NM_005896	Isocitrate dehydrogenase 1 (NADP+), soluble	IDH1	3.94	1.50	0.38
6	NM_020307	Cyclin L1	CCNL1	3.93	1.48	0.38
7	NM_001067	Topoisomerase (DNA) II α, 170 kDa	TOP2A	3.84	1.00	0.26
8	NM_004830	Cofactor required for Sp1 transcriptional activation 3	CRSP3	3.76	0.69	0.18
9	Y10183	Activated leukocyte cell adhesion molecule	ALCAM	3.65	1.14	0.31
10	AL110194	High-mobility group box 1	HMGB1	3.58	1.77	0.50
11	NM_016343	Centromere protein F, 350/400ka (mitosin)	CENPF	3.57	1.12	0.31
12	NM_005077	Transducin-like enhancer of split 1	TLE1	3.47	1.26	0.36
13	NM_006024	Tax1 binding protein 1	TAX1BP1	3.45	0.66	0.19
14	NM_015906	Tripartite motif-containing 33	TRIM33	3.41	1.42	0.41
15	AL117665	Gem (nuclear organelle) associated protein 5	GEMIN5	3.41	1.61	0.47
16	NM_016142	Hydroxysteroid (17-ß) dehydrogenase 12	HSD17B12	3.40	1.60	0.47
17	AL161973	Myeloid/lymphoid or mixed-lineage leukemia	MLLT4	3.40	1.26	0.37
18	NM_003850	Succinate-CoA ligase, ADP-forming	SUCLA2	3.36	1.15	0.34
19	BC014003	Coiled-coil domain containing 5	CCDC5	3.35	1.30	0.39
20	AL157438	Mitogen-activated protein kinase 1	MAPK1	3.29	1.17	0.36
21	AK023345	Diaphanous homolog 1 (*Drosophila*)	DIAPH1	3.25	0.81	0.25
22	BF571514	Dual specificity phosphatase 10	DUSP10	3.14	0.75	0.24
23	NM_000366	Tropomyosin 1 (α)	TPM1	3.11	1.43	0.46
24	NM_006793	Peroxiredoxin 3	PRDX3	3.10	0.82	0.26
25	AL162068	Nucleosome assembly protein 1-like 1	NAP1L1	3.08	1.30	0.42

Table V. Continued.

A. At 3 h.

	GenBank	Name	Symbol	Rank	HepG2	Hep3B
26	AF305083	Fucosyltransferase 4	FUT4	3.03	1.17	0.39
27	NM_005928	Milk fat globule-EGF factor 8 protein	MFGE8	3.01	1.05	0.35
28	NM_002166	Inhibitor of DNA binding 2	ID2	3.00	1.38	0.46
29	AB023195	Additional sex combs like 1 (*Drosophila*)	ASXL1	0.33	0.52	1.57
30	AB020711	CDC2-related protein kinase 7	CRK7	0.33	0.41	1.25
31	NM_014970	Kinesin-associated protein 3	KIFAP3	0.33	0.54	1.63
32	AL080157	WD repeat domain 21	WDR21	0.33	0.52	1.58
33	BG427872	Ubiquitin specific protease 46	USP46	0.33	0.52	1.57
34	AF208850	Protein tyrosine phosphatase type IVA2	PTP4A2	0.33	0.47	1.44
35	AL512725	Midnolin	MIDN	0.33	0.43	1.32
36	BE888791	Hepatocellular carcinoma related protein 1	FLJ32642	0.33	0.39	1.20
37	AA400519	Polyhomeotic like 3 (*Drosophila*)	PHC3	0.32	0.37	1.14
38	NM_021170	bHLH factor Hes4	Hes4	0.32	0.45	1.41
39	NM_014501	Ubiquitin-conjugating enzyme E2S	UBE2S	0.32	0.40	1.27
40	NM_014595	5', 3'-nucleotidase, cytosolic	NT5C	0.31	0.37	1.17
41	D30612	Zinc finger protein 282	ZNF282	0.31	0.49	1.55
42	BC001043	Splicing factor 4	SF4	0.31	0.42	1.36
43	NM_018688	Bridging integrator 3	BIN3	0.31	0.41	1.35
44	NM_001439	Exostoses (multiple)-like 2	EXTL2	0.30	0.46	1.53
45	BG340148	HESB-like domain containing 1	HBLD1	0.30	0.45	1.52
46	NM_005186	Calpain 1, (mu/I) large subunit	CAPN1	0.30	0.38	1.30
47	NM_003739	Aldo-keto reductase 1C3	AKR1C3	0.29	0.47	1.60
48	NM_002673	Plexin B1	PLXNB1	0.29	0.44	1.53
49	NM_007246	Kelch-like 2, Mayven (*Drosophila*)	KLHL2	0.29	0.50	1.73
50	NM_002226	Jagged 2	JAG2	0.28	0.46	1.61
51	NM_002661	Phospholipase C, γ 2	PLCG2	0.28	0.48	1.72
52	NM_012116	Cas-Br-M ecotropic retroviral transforming C	CBLC	0.27	0.45	1.63
53	NM_001703	Brain-specific angiogenesis inhibitor 2	BAI2	0.27	0.50	1.85
54	BF115152	G protein-coupled receptor 110	GPR110	0.27	0.37	1.36
55	NM_000819	GART	GART	0.27	0.54	2.02
56	AF207881	Cytosolic ovarian carcinoma antigen 1	COVA1	0.26	0.55	2.16
57	NM_012164	F-box and WD-40 domain protein 2	FBXW2	0.24	0.17	0.68
58	NM_015953	Nitric oxide synthase interacting protein	NOSIP	0.24	0.43	1.80
59	BG254520	MADS box transcription enhancer factor 2A	MEF2A	0.23	0.33	1.44
60	NM_005652	Telomeric repeat binding factor 2	TERF2	0.22	0.39	1.79
61	AF277181	PNAS-123	LOC85028	0.22	0.37	1.71
62	NM_003686	Exonuclease 1	EXO1	0.19	0.20	1.05
63	NM_005873	Regulator of G-protein signalling 19	RGS19	0.19	0.34	1.80
64	D87452	Inositol hexaphosphate kinase 1	IHPK1	0.18	0.48	2.72
65	NM_016434	Tumor necrosis factor receptor superfamily 6b	TNFRSF6B	0.15	0.54	3.64

B. At 24 h.

	GenBank	Name	Symbol	Rank	HepG2	Hep3B
1	NM_014426	Sorting nexin 5	SNX5	11.70	2.39*	0.20
2	NM_003457	Zinc finger protein 207	ZNF207	9.91	1.80	0.18
3	NM_018477	Actin-related protein 10 homolog	ACTR10	9.36	1.10	0.12
4	NM_006302	Glucosidase I	GCS1	9.17	3.26	0.36
5	AW978861	Lysophospholipase-like 1	LYPLAL1	8.14	0.71	0.09
6	NM_000521	Hexosaminidase B (ß polypeptide)	HEXB	7.98	2.18	0.27

Continued.

	GenBank	Name	Symbol	Rank	HepG2	Hep3B
7	X92518	High mobility group AT-hook 2	HMGA2	7.85	1.42	0.18
8	NM_006142	Stratifin	SFN	7.76	0.69	0.09
9	NM_005608	Protein tyrosine phosphatase	PTPRCAP	7.63	1.64	0.21
10	NM_002467	V-myc	MYC	7.19	0.89	0.12
11	NM_005381	Nucleolin	NCL	6.90	1.57	0.23
12	BI092644	SEC22 vesicle trafficking protein-like 1	SEC22L1	6.49	1.24	0.19
13	AB067486	Tubulin, γ complex associated protein 5	TUBGCP5	6.16	1.45	0.24
14	NM_000534	PMS1 postmeiotic segregation increased 1	PMS1	6.02	1.30	0.22
15	D31763	Zinc finger protein 33a (KOX 31)	ZNF33A	5.71	1.72	0.30
16	NM_003072	SMARCA4	SMARCA4	5.56	1.43	0.26
17	NM_002600	Phosphodiesterase 4B, cAMP-specific	PDE4B	4.99	1.40	0.28
18	AK025550	Sprouty-related, EVH1 domain containing 2	SPRED2	4.98	1.78	0.36
19	AB051532	Semaphorin 4B	SEMA4B	4.60	1.34	0.29
20	NM_013400	Replication initiator 1	REPIN1	4.59	0.72	0.16
21	NM_006554	Metaxin 2	MTX2	4.58	1.53	0.33
22	NM_000034	Aldolase A, fructose-bisphosphate	ALDOA	4.54	1.61	0.35
23	NM_005985	Snail homolog 1 (*Drosophila*)	SNAI1	4.39	0.67	0.15
24	NM_016453	SH3 protein interacting with Nck, 90 kDa	AF3P21	4.31	1.52	0.35
25	NM_014623	Male-enhanced antigen	MEA	4.29	1.57	0.36
26	NM_030805	Lectin, mannose-binding 2-like	LMAN2L	4.20	1.35	0.32
27	NM_001407	Cadherin, EGF LAG seven-pass G-type receptor 3	CELSR3	4.15	1.25	0.30
28	NM_004708	Programmed cell death 5	PDCD5	4.03	1.11	0.27
29	NM_024518	UL16 binding protein 3	ULBP3	3.97	1.00	0.25
30	AK026320	Polymeric immunoglobulin receptor	PIGR	3.97	1.04	0.26
31	NM_031902	Mitochondrial ribosomal protein S5	MRPS5	3.85	0.73	0.19
32	NM_004960	Fusion	FUS	3.79	1.45	0.38
33	NM_003031	Seven in absentia homolog 1 (*Drosophila*)	SIAH1	3.79	0.99	0.26
34	NM_005095	Zinc finger protein 262	ZNF262	3.71	0.96	0.26
35	NM_005863	Neuroepithelial cell transforming gene 1	NET1	3.69	0.28	0.08
36	NM_005863	Neuroepithelial cell transforming gene 1	NET1	3.69	0.28	0.08
37	AI653251	Benzodiazapine receptor associated protein 1	BZRAP1	3.60	1.33	0.37
38	NM_004998	Myosin IE	MYO1E	3.52	1.47	0.42
39	NM_022900	O-acetyltransferase	CAS1	3.50	0.54	0.16
40	NM_007265	Suppressor of *S. cerevisiae* gcr2	HSGT1	3.33	1.15	0.35
41	AB023190	Syntrophin associated serine/threonine kinase	SAST	3.33	1.37	0.41
42	AK026745	Nuclear protein double minute 1	MDM1	3.31	1.25	0.38
43	NM_031457	Membrane-spanning 4-domains A 8B	MS4A8B	3.22	0.98	0.31
44	AL536811	Actinfilin	LOC339451	3.21	0.98	0.31
45	NM_012289	Kelch-like ECH-associated protein 1	KEAP1	3.20	1.42	0.44
46	NM_016131	RAB10, member RAS oncogene family	RAB10	3.11	1.29	0.42
47	NM_002435	Mannose phosphate isomerase	MPI	3.05	1.23	0.40
48	NM_012179	F-box only protein 7	FBXO7	3.00	1.32	0.44
49	BC002971	Chaperonin containing TCP1, subunit 5	CCT5	0.33	0.39	1.20
50	NM_002999	Syndecan 4 (amphiglycan, ryudocan)	SDC4	0.29	0.42	1.45
51	NM_020167	Neuromedin U receptor 2	NMU2R	0.29	0.47	1.64
52	NM_005471	Glucosamine-6-phosphate deaminase 1	GNPDA1	0.26	0.24	0.94
53	NM_002276	Keratin 19	KRT19	0.24	0.45	1.84
54	NM_005143	Haptoglobin	HP	0.24	0.52	2.16
55	NM_032593	Histidine triad nucleotide binding protein 2	HINT2	0.15	0.47	3.15
56	BC012778	Calmodulin regulated spectrin-associated protein 1	CAMSAP1	0.04	0.49	13.15

Table V. Continued.

C. At 72 h.

	GenBank	Name	Symbol	Rank	HepG2	Hep3B
1	BC010464	Mitogen-activated protein kinase kinase kinase 3	MAP3K3	5.17	2.09*	0.40
2	NM_001703	Brain-specific angiogenesis inhibitor 2	BAI2	4.35	1.61	0.37
3	NM_014905	Glutaminase	GLS	3.60	1.34	0.37
4	AK023115	Transcriptional coactivator tubedown-100	TBDN100	3.51	1.52	0.43
5	NM_018841	Guanine nucleotide binding protein γ 12	GNG12	3.32	1.23	0.37
6	NM_021724	Nuclear receptor subfamily 1, group D, member 1	NR1D1	3.28	1.24	0.38
7	NM_001311	Cysteine-rich protein 2	CRIP2	3.21	1.17	0.36
8	NM_002090	Chemokine (C-X-C motif) ligand 3	CXCL3	3.20	1.35	0.42
9	NM_002630	Progastricsin (pepsinogen C)	PGC	3.17	1.13	0.35
10	NM_015833	Adenosine deaminase, RNA-specific, B1	ADARB1	3.10	1.17	0.38
11	NM_005121	Thyroid hormone receptor-associated protein	TRAP240	3.08	1.34	0.44
12	NM_000900	Matrix Gla protein	MGP	3.05	1.11	0.37
13	BF115152	G protein-coupled receptor 110	GPR110	3.03	0.65	0.22
14	NM_014879	G protein-coupled receptor 105	GPR105	3.02	0.65	0.21
15	NM_005180	COMM domain containing 3	COMMD3	0.33	0.48	1.46
16	NM_016000	tRNA nucleotidyl transferase, CCA-adding, 1	TRNT1	0.33	0.50	1.52
17	NM_005113	Golgi autoantigen, golgin subfamily a, 5	GOLGA5	0.33	0.54	1.63
18	NM_005826	Heterogeneous nuclear ribonucleoprotein R	HNRPR	0.33	0.43	1.30
19	NM_002940	ATP-binding cassette, sub-family E1	ABCE1	0.33	0.48	1.46
20	NM_005880	DnaJ (Hsp40) homolog, subfamily A, member 2	DNAJA2	0.33	0.49	1.51
21	AB032967	Zinc finger protein 473	ZNF473	0.33	0.45	1.38
22	AK001555	RAB18, member RAS oncogene family	RAB18	0.33	0.51	1.57
23	L39061	TATA box binding protein (TBP)-associated factor	TAF1B	0.32	0.26	0.82
24	NM_003664	Adaptor-related protein complex 3, ß 1	AP3B1	0.32	0.43	1.33
25	NM_006756	Transcription elongation factor A (SII), 1	TCEA1	0.32	0.41	1.29
26	BI094140	Leucine-rich PPR-motif containing	LRPPRC	0.32	0.54	1.71
27	NM_003076	SMARCD1	SMARCD1	0.31	0.44	1.39
28	NM_032704	Tubulin α 6	TUBA6	0.31	0.33	1.08
29	BC000143	Engulfment and cell motility 2	ELMO2	0.31	0.50	1.61
30	AL545781	Mitochondrial ribosomal protein L10	MRPL10	0.30	0.31	1.02
31	NM_001316	CSE1 chromosome segregation 1-like	CSE1L	0.30	0.41	1.34
32	NM_004060	Cyclin G1	CCNG1	0.30	0.54	1.80
33	NM_003715	Vesicle docking protein p115	VDP	0.29	0.38	1.32
34	NM_004539	Asparaginyl-tRNA synthetase	NARS	0.29	0.34	1.17
35	NM_003589	Cullin 4A	CUL4A	0.29	0.44	1.54
36	X68560	Sp3 transcription factor	SP3	0.27	0.46	1.72
37	NM_003318	TTK protein kinase	TTK	0.27	0.51	1.90
38	NM_003299	Tumor rejection antigen (gp96) 1	TRA1	0.26	0.37	1.39
39	NM_014750	Discs, large homolog 7 (*Drosophila*)	DLG7	0.26	0.41	1.56
40	NM_012484	Hyaluronan-mediated motility receptor	HMMR	0.26	0.40	1.52
41	AB032945	Myosin VB	MYO5B	0.26	0.49	1.87
42	NM_005030	Polo-like kinase 1 (*Drosophila*)	PLK1	0.25	0.38	1.50
43	NM_007202	A kinase (PRKA) anchor protein 10	AKAP10	0.25	0.47	1.85
44	NM_005733	Kinesin family member 20A	KIF20A	0.25	0.53	2.14
45	NM_021249	Sorting nexin 6	SNX6	0.24	0.35	1.45
46	NM_031217	Kinesin family member 18A		0.24	0.48	2.02
47	NM_006392	Nucleolar protein 5A	NOL5A	0.23	0.21	0.91
48	NM_003136	Signal recognition particle, 54 kDa	SRP54	0.23	0.42	1.84
49	NM_003905	Amyloid ß precursor protein binding protein 1	APPBP1	0.23	0.50	2.22

Continued.

	GenBank	Name	Symbol	Rank	HepG2	Hep3B
50	NM_018492	T-LAK cell-originated protein kinase	TOPK	0.22	0.41	1.82
51	NM_004766	Coatomer protein complex 2	COPB2	0.22	0.44	2.00
52	NM_003392	Wingless-type MMTV integration site family 5A	WNT5A	0.22	0.19	0.88
53	NM_007355	Heat shock 90 kDa protein 1, ß	HSPCB	0.19	0.22	1.12
54	NM_016343	Centromere protein F, 350/400ka (mitosin)	CENPF	0.19	0.43	2.30
55	NM_005701	RNA, U transporter 1	RNUT1	0.18	0.28	1.56
56	NM_001067	Topoisomerase (DNA) II α, 170 kDa	TOP2A	0.13	0.34	2.63

From the top 200 down-regulated genes in HepG2 or Hep3B cells presented in the legend of Table II, we selected differentially down-regulated genes and then ranked their orders by dividing the expression ratios of the radiation-responsive genes of HepG2 cells by those of Hep3B cells. The rank >3 or <0.3 indicates higher or lower levels of gene expression in HepG2 cells compared to those of Hep3B cells, respectively. [a]Intensity ratios of Cy5/Cy3.

Table VI. Association of DNA repair gene family with radiation sensitivity.

			Time after radiation					
			HepG2			Hep3B		
GenBank	Name	Symbol	3 h	24 h	72 h	3 h	24 h	72 h
BER								
AL117459	RAD51-like 3 (*S. cerevisiae*)	RAD51L3	1.10[a]	0.42	1.17	**1.62**	0.36	0.52
NM_002434	N-methylpurine-DNA glycosylase	MPG	0.80	0.89	1.18	1.47	0.81	0.68
NM_003362	Uracil-DNA glycosylase 1	UNG1	1.19	0.94	0.71	0.75	0.54	0.92
NM_003925	Methyl-CpG binding domain protein 4	MBD4	0.91	0.93	0.72	0.78	1.18	1.26
NM_016819	8-oxoguanine DNA glycosylase	OGG1	0.54	0.58	1.03	0.60	0.98	0.81
NM_021147	Uracil-DNA glycosylase 2	UNG2	0.90	1.25	0.67	0.52	1.04	1.07
DSB								
NM_001469	Thyroid autoantigen, 70 kDa (Ku antigen)	G22P1	1.11	**2.54**	0.65	0.66	1.40	1.11
NM_002485	Nijmegen breakage syndrome 1 (nibrin)	NBS1	1.05	0.97	0.89	1.12	0.61	0.97
NM_006265	RAD21 homolog (*S. pombe*)	RAD21	0.80	0.76	0.76	0.61	**2.65**	1.30
U47077	DNA-Pkcs	PRKDC	1.48	**1.96**	1.25	0.84	1.07	1.22
U63139	RAD50 homolog (*S. cerevisiae*)	RAD50	0.86	0.78	0.55	0.81	1.32	1.03
NER								
NM_000123	ERCC, complementation group 5	ERCC5	0.73	**1.98**	1.00	0.37	0.98	**1.94**
NM_001983	ERCC, complementation group 1	ERCC1	0.85	1.09	0.89	1.01	1.15	0.49
NM_002874	RAD23 homolog B (*S. cerevisiae*)	RAD23B	1.37	1.20	1.10	0.71	1.00	1.13
NM_005053	RAD23 homolog A (*S. cerevisiae*)	RAD23A	0.60	0.51	0.56	0.75	1.04	0.57
NM_020196	XPA binding protein 2	XAB2	1.35	1.22	1.17	0.80	1.28	1.16
NM_022362	MMS19-like	MMS19L	1.35	1.43	1.30	1.13	0.80	0.88
MMR								
AB037834	Nedd4 binding protein 2	N4BP2	**2.10**	**1.54**	1.73	1.00	**1.91**	1.31
AF319566	Three prime repair exonuclease 1	TREX1	0.61	0.47	0.58	1.47	0.71	0.34
AJ245661	MutS homolog 5 (*E. coli*)	MSH5	1.13	1.14	**1.58**	1.05	0.70	0.96
NM_000179	MutS homolog 6 (*E. coli*)	MSH6	**3.67**	1.16	**2.16**	0.98	1.36	1.14
NM_000249	MutL homolog 1	MLH1	1.04	1.24	0.46	0.70	1.25	1.27
NM_000251	MutS homolog 2	MSH2	0.94	0.88	1.00	0.71	1.38	0.88
NM_000534	Postmeiotic segregation increased 1	PMS1	1.00	1.30	0.63	**1.85**	0.22	0.76

A family of DNA repair genes was selected using the gene ontology algorithm, then classified into four categories: base excision repair (BER), double-strand break repair (DSB), nucleotide excision repair (NER), and mismatch repair (MMR). [a]Intensity ratios of Cy5/Cy3. Bold represents expression ratios >1.5-fold.

Discussion

The development of microarray technology for large-scale analysis of gene expression makes it possible to systemically identify molecular markers of human cancers (14). In the analyses of HCC gene expression profiles, classifier genes in most cases have also been evaluated for the discrimination of HCC and prediction of their clinical outcomes (15-19). In the present study, we performed temporal analysis of radiation-responsive genes and subsequently identified the radiation sensitivity classifier in HCC. The most notable finding was that greatly different subsets of genes were sequentially up- or down-regulated during the period of 72 h post-irradiation. This finding indicates that HCC cells, upon exposure to radiation, markedly and differently activate or deactivate genes between early and late phases after irradiation. When radiation-responsive genes were classified according to their function using gene ontology, we found that DNA repair-associated genes were well matched with radiation sensitivity among other classes of physiologically relevant genes, and the inability to repair double-strand breaks induced by radiation may cause cell death (20,21). In the present study, relatively radiation-resistant HepG2 cells showed up-regulation of a substantial fraction of DNA repair-associated genes, including Ku70, PMS1, MSH6, DNA-dependent protein kinase, and ERCC5 genes. On the other hand, relatively radiation-sensitive Hep3B cells exhibited the above DNA repair-associated genes as unchanged or down-regulated. Inactivation of these DNA repair-associated genes has been shown to decrease the survival rate of cells upon exposure to DNA damage-inducing drugs, thereby sensitizing cells to anticancer agents such as radiation (22). These earlier findings, together with our present result that DNA repair-associated genes correlate with radiation sensitivity of HCC cells much more strongly than any other physiological processes, indicate that differential radiation sensitivity of HCC cells may be due to their repair capacity of DNA strand breaks.

Acknowledgements

This work was supported by a grant from National Nuclear R & D program and Human Genome Project (FG03-0601-003-1-0-0), Korean Ministry of Science and Technology. Work in the lab of M.H.C. was supported by the grant from Nano Systems Institute at National Core Research Center (NSI-NCRC) through Korea Science and Engineering Foundation (KOSEF). Work in the lab of K.Y.C. was partly supported by the Korea Research Foundation Grant (# KRF-2003-015-C00400) from the Korea Ministry of Science.

References

1. Qin LX and Tang ZY: The prognostic significance of clinical and pathological features in hepatocellular carcinoma. World J Gastroenterol 8: 193-199, 2002.
2. Mathurin P, Rixe O, Carbonell N, Bernard B, Cluzel P, Bellin MF, Khayat D, Opolon P and Poynard T: Review article: overview of medical treatments in unresectable hepatocellular carcinoma-an impossible meta-analysis? Aliment Pharmacol Ther 12: 111-126, 1998.
3. Garcea G, Lloyd TD, Aylott C, Maddern G and Berry DP: The emergent role of focal lover ablation techniques in the treatment of primary and secondary liver tumours. Eur J Cancer 39: 2150-2164, 2003.
4. Scheele J, Stang R, Altendorf-Hofmann A and Paul M: Resection of colorectal liver metastases. World J Surg 19: 59-71, 1995.
5. Park HC, Seong J, Han KH, Chon CY, Moon YM and Suh CO: Dose-response relationship in local radiotherapy for hepatocellular carcinoma. Int J Radiation Oncology Biol Phys 54: 150-155, 2002.
6. Cochrane AMG, Murray-Lyon IM, Brinkley DM and Williams R: Quadruple chemotherapy versus radiotherapy in treatment of primary hepatocellular carcinoma. Cancer 40: 609-614, 1977.
7. Ingold JA, Reed GB, Kaplan HS and Bagshaw MA: Radiation hepatitis. Am J Roentgenol Radium Ther Nucl Med 93: 200-208, 1965.
8. Llovet JM, Bustamante J, Castells A, Vilana R, Ayuso M, Sala M, Bru C, Rodes J and Bruix J: Natural History of untreated nonsurgical heptocellular carcinoma: Rationale for the design and evaluation of therapeutic trials. Hepatology 29: 62-67, 1999.
9. Yang YH, Dudoit S, Luu P, Lin DM, Peng V, Ngai J and Speed TP: Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. Nucleic Acids Res 30: e15, 2002.
10. Graveel CR, Jatkoe T, Madore SJ, Holt AL and Farnham PJ: Expression profiling and identification of novel genes in hepatocellular carcinomas. Oncogene 20: 2074-2712, 2001.
11. Al-Shahrour F, Diaz-Uriarte R and Dopazo J: FatiGO: a web tool for finding significant associations of gene ontology terms with groups of genes. Bioinformatics 20: 578-580, 2004.
12. Drotschmann K, Topping RP, Clodfelter JE and Salsbury FR: Mutations in the nucleotide-binding domain of MutS homologs uncouple cell death from cell survival. DNA Repair 3: 729-742, 2004.
13. Hall MC, Shcherbakova PV, Fortune JM, Borchers CH, Dial JM, Tomer KB and Kunkel TA: DNA binding by yeast Mlh1 and Pms1: implications for DNA mismatch repair. Nucleic Acids Res 31: 2025-2034, 2003.
14. Kawai HF, Kaneko S, Honda M, Shirota Y and Kobayashi K: α-Fetoprotein-producing hepatoma cell lines share common expression profiles of genes in various categories demonstrated by cDNA microarray analysis. Hepatology 33: 676-691, 2001.
15. Okabe H, Satoh S, Kato T, Kitahara O, Yanagawa R, Yamaoka Y, Tsunoda T, Furukawa Y and Nakamura Y: Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: Identification of genes involved in viral carcinogenesis and tumor progression. Cancer Res 61: 2129-2137, 2001.
16. Chen X, Cheung ST, So S, Fan ST, Barry C, Higgins J, Lai KM, Ji J, Dudoit S, Ng IO, Van de Rijn M, Botstein D and Brown PO: Gene expression patterns in human liver cancers. Mol Biol Cell 13: 1929-1939, 2002.
17. Iizuka N, Oka M, Yamada-Okabe H, Mori N, Tamesa T, Okada T, Takemoto N, Tangoku A, Hamada K, Nakayama H, Miyamoto T, Uchimura S and Hamamoto Y: Comparison of gene expression profiles between hepatitis B virus- and hepatitis C virus infected hepatocellular carcinoma by oligonucleotide microarray data on the basis of a supervised learning method. Cancer Res 62: 3939-3944, 2002.
18. Iizuka N, Oka M, Yamada-Okabe H, Nishida M, Maeda Y, Mori N, Takao T, Tamesa T, Tangoku A, Tabuchi H, Hamada K, Nakayama H, Ishitsuka H, Miyamoto T, Hirabayashi A, Uchimura S and Hamamoto Y: Oligonucleotide microarray for prediction of early intrahepatic recurrence of hepatocellular carcinoma after curative resection. Lancet 361: 923-929, 2003.
19. Kim BY, Lee JG, Park SH, Ahn JY, Ju YJ, Chung JH, Han CJ, Jeong SH, Yeom YI, Kim SS, Lee YS, Kim CM, Eom EM, Lee DH, Choi KY, Cho MH, Suh KS, Choi DW and Lee KH: Feature genes of hepatitis B virus-positive hepatocellular carcinoma, established by its molecular discrimination approach using prediction analysis of microarray. Biochim Biophys Acta 1739: 50-61, 2004.
20. Frankenberg-Schwager M, Frankenberg D, Blocher D and Adamczyk C: Repair of DNA double-strand breaks in irradiated yeast cells under nongrowth conditions. Radiat Res 82: 498-510, 1980.
21. Blocher D and Pohlit W: DNA double strand breaks in Ehrlich ascites tumour cells at low doses of x-rays. II. Can cell death be attributed to double strand breaks? Int J Radiat Biol Relat Stud Phys Chem Med 42: 329-338, 1982.
22. Robson T, Price ME, Moore ML, Joiner MC, McKeown SR, McKelvey-Martin VJ and Hirst DG: Increased repair and cell survival in cells treated with DIR1 antisense oligonucleotides: implications for induced radioresistance. Int J Radiat Biol 76: 617-623, 2000.