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▣ 論 文 ▣

Gene Expression Patterns of Spleen, Lung and Brain with Different Radiosensitivity in C57BL6 Mice

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Abstract - Although little information is available on the underlying mechanisms, various genetic factors have been associated with tissue-specific responses to radiation. In the present study, we explored the possibility whether organ specific gene expression is associated with radiosensitivity using samples from brain, lung and spleen. We examined intrinsic expression pattern of 23 genes in the organs by semi-quantitative RT-PCR method using both male and female C57BL/6 mice. Expression of p53 and p21, well known factors for governing sensitivity to radiation or chemotherapeutic agents, was not different among the organ types. Both higher expression of sialyltransferase, delta7-sterol reductase, leptin receptor splice variant form 12.1, and Cu/Zn superoxide dismutase (SOD) and lower expression of alphaB crystalline were specific for spleen tissue. Expression level of glutathione peroxidase and APO-1 cell surface antigen gene in lung tissue was high, while that of Na, K-ATPase alpha-subunit, Cu/ZnSOD, and cyclin G was low. Brain, radioresistant organ, showed higher expressions of Na, K-ATPase-subunit, cyclin G, and nucleolar protein hNop56 and lower expression of delta7-sterol reductase. The result revealed a potential correlation between gene expression patterns and organ sensitivity, and identified genes which might be responsible for organ sensitivity.

Key words : Organ specific genes, Radiosensitivity, Semi-quantitative RT-PCR

Introduction

Cellular and tissue sensitivity against ionizing radiation depends on endogenous gene expression. It has been known that tissue or cells show different response to various stimuli, such as ionizing radiation, depending on their genetic background and on the decision whether the damage is dealt with by apoptosis, rescue or repair. Death of individual cells removes the problem from the tissue. However, if the cell does not die, it may acquire genomic instability and lead to a population of cells with abnormally high susceptibility to chromosomal instability, mutation, and other delayed effects [1-5].

Studies with inbred strains of rodents have clearly shown genotype-dependent differences in response to radiation exposure, including susceptibility to radiation-induced cell death, cellular transformation and tumor formation, as well as differences in susceptibility to radiation-induced chromosomal instability [6-9]. Among various experimental systems, mouse models have proven to be very useful in identifying genes that modify radiation sensitivity. For instance, p53 deficient mice were influenced by stress response such as radiation [10-12]. Furthermore, a particular type of tumor that arises is dependent on the genetic background [13-16].

Understanding of the variation in the radiation response at the level of cell, tissue and human being is important in determining the potentially harmful effects of environmental, accidental, or therapeutic radiation exposure [17-19]. However, the nature of these factors is largely unknown. *In vitro* studies cannot reveal the complexity of tissue response, where different cell types and cells at different stages of differentiation/ activation show markedly different responses to radiation damage [20].

Whole gene expression profiling has become one of the most widely used approaches to identify genes and their functions in the context of specific biological questions. There is growing acknowledgement of the usefulness of determining expression patterns to identify and categorize genes, be it to use as disease markers, to discover drug targets, to map specific pathways, or to find markers of drug toxicity in early drug testing [21-23]. The mRNA of human myeloid cancer cell lines has been quantified to define stress response gene such as p21CIP1/ WAF1 and GADD45 at doses of gamma rays between 2 and 50 cGy [24], and ICAM-3 has been suggested as a biomarker to predict the radiation resistance in cervical cancer [25]. Furthermore, two mitochondrial ATP synthases are overexpressed in human differentiated keratinocytes by radiation [26]. Previously, we identified 44 genes in human peripheral blood lymphocytes (PBL) whose expressions were increased by ionizing radiation using microarray analysis [27]. Their cellular functions include cell cycle regulation, redox regulation, and cell surface receptors among many others. However, no attempt has been made to examine exact roles of single gene or genes in combination, particularly in correlation with radiation sensitivity. In this study, to identify the genes which might regulate radiation sensitivity of tissue, we analyzed expression of 23 genes which were selected from 44 genes in microarray data of irradiated PBL. We analyzed gene expression pattern in mouse organs including brain, spleen and lung, which have different intrinsic radiosensitivity. The result revealed a potential correlation between gene expression patterns and organ sensitivity, and identified genes which might be responsible for organ sensitivity.

MATERIALS AND METHODS

Animals

C57BL/6 female and male mice, 6-7 weeks old, were purchased from Charles River Japan Inc. and were kept in clean conventional environment. The housing conditions were 22±2°C, 50±10% humidity, and a 12 hr light-dark cycle. Studies were conducted under guidelines for the use and care of laboratory animals and were approved by the Institutional Animal Care and Use Committee of the Korea Institute Radiological and Medical Sciences (KIRAMS).

Irradiation

Ten or 0.2Gy of whole body irradiation for individual mouse was performed by using ^{137}Cs gamma-ray source (Atomic Energy of Canada, Ltd., Ontario, Canada) with dose rate of 3.81 Gy/min. Sham exposed mice were used as control mice.

Tissue preparation

One or three days after irradiation, animals were immediately sacrificed by cervical dislocation. Lung, spleen and brain tissues were quickly harvested from the mice. Some of each tissue were fixed in 4% paraformaldehyde overnight at 4°C and paraffin embedded for hematoxylin and eosin (H/E) staining. Lung, spleen and brain tissues from unirradiated mice were preserved in liquid nitrogen tank for RNA preparation.

TUNEL assay

The TUNEL assay was performed by using the ApopTag Plus Peroxidase *in Situ* Apoptosis Detection Kit (Intergen, Purchase, NY, USA) following the protocols provided by the manufacturer.

Semiquantitative RT-PCR

Aliquots of 0.5 μg of total RNA from spleen, lung and brain of each mouse were isolated with TRI reagent (MRC, Cincinnati, OH, USA) according to the manufacturer's instructions. The reaction mixture contained 2 μl of 10xRT buffer (QIAGEN), 2μl of 5 mM dNTPs (QIAGEN), 1 μl of 10 U/ml RNAsin (Promega), 2 μl of 10 μM oligo (dT)-15 primer, 1.25 μg of total RNA, and 1 μl of Omniscript reverse transcriptase (QIAGEN) in a final volume of 20 μl. The mixture was incubated at 37°C for 1 hr. The transcription reaction was terminated by heating the mixture at 95°C for 5 min and then chilling it on ice. PCR cycles were 32 for all the reaction. Primer sequences are listed in Table 2. The PCR products were analyzed by 2% agarose gel electrophoresis and stained with ethidium bromide. Quantification was carried out using an image analyzer with the MCID software program (Image Research Iic., Ontario, Canada).

RESULTS

No animal died after whole body irradiation until mice were autopsied. Through previous microarray experiment, we identified 44 genes in human PBL that were upregulated by radiation [27]. We performed semi-quantitative RT-PCR of all 44 genes in the 3 organs using mouse primers, however, only 23 genes were selected because these genes were detected in at least one of 3 organs (Tables 1 and 2).

Table 1. Gene list used in RT-PCR analysis.

	Gene bank accession no	Gene name	Function
1	d00632	Glutathione peroxidase	Antioxidant enzyme
2	y12781	Transducin (beta) like 1 protein	Control of differentiation
3	x63717	APO-1 cell surface antigen	Regulation of cell death
4	u02680	Protein tyrosine kinase	Tyrosine phosphorylation
5	x53586	Integrin alpha 6	Regulating cell growth
6	j02764	Platelet membrane glycoprotein Iib (ITGA2B)	Cell surface molecule
7	p34932	HSP70	Chaperone function
8	x17247	Sialyltransferase	Glycosyltransferase
9	m85234	Nuclease sensitive element binding protein-1	Transcription regulation
10	x54937	Cannabinoid receptor	G protein coupled receptor family
11	l42176	DRAL	Small GTPase superfamily
12	u32315	Syntaxin 3	Membrane transporter
13	x04297	Na, K-ATPase alpha-subunit	Ion regulation
14	af034544	Delta7-sterol reductase	7-Dehydrocholesterol reductase activity
15	s69022	Myosin light chain-2	Calcium binding protein
16	u72391	Neogenin	Cell surface receptor
17	u66496	Leptin receptor splice variant form 12.1	Cell surface receptor
18	x02317	Cu/Zn superoxide dismutase	Antioxidant enzyme
19	m15796	Cyclin protein gene	Cell cycle regulation
20	u53328	Cyclin G	Cell cycle regulation
21	y12065	Nucleolar protein hNop56	Pre-rRNA processing
22	af001601	Paraoxonase (PON2)	A-oxonase family
23	nm00185	aB Crystaline	Chaperone function

Table 2. PCR primer sequences.

	Gene bank accession no	Left Primer	Right Primer
1	d00632	CATCCCATGTCCACCATGTA	AGCTGGTTTTTCCTTTGGGT
2	y12781	ACCAATGGAACACTCTTGGC	GCCTGCACACATGGATACAC
3	x63717	ATAAGCCCTGTCCTCCAGGT	GACAAAGCCACCCCAAGTTA
4	u02680	GGAGGTGGCCACATTAAAGA	GCATGTGTATCCAGGCATTG
5	x53586	ATCCGGAAATATGGAGACCC	ACTCAGGACATAACACCGCC
6	j02764	AGGTGAGAGGGAGCAGAACA	GCAGCACAAACTGATCCAGA
7	p34932	TTCCGTTTCCAGCCCCCAATC	CGTTGAGCCCCGCGATGTACA
8	x17247	GAAGCAGGCTTGTTTTCCTG	TCTGCACTGAACTTGATGCC
9	m85234	AGGGAGAAGTGATGGAGGGT	GGTAAGCCGGCATTTACTCA
10	x54937	CTGAGGAGTAAGGACCTGCG	TGACATGTGGCAATCACCTT
11	l42176	GAAGTGCTCCCTCTCACTGG	ATTTGGGTGTGCCTTACTCG
12	u32315	CATCAAGGAGCTTCACGACA	AAGAGCTTTCCCAAGTGCAA
13	x04297	GACTGGTGACGGTGTGAATG	TGCTCATAGGTCCACTGCTG
14	af034544	CAACCACCAGAAGGACCTGT	GGCAAAAGCAAGGAACAGAG
15	s69022	ACAGGGATGGCTTCATTGAC	GATCTGCAAAGACAGAGCCC
16	u72391	ACCCCAGCCTGTGATTAGTG	TGTGATGGTTCAGAGCTTGC
17	u66496	TCCCATATCTGAGCCCAAAG	CATCAGGGGCTTCCAAAGTA
18	x02317	AGGGCATCATCAATTTCGAG	TCTTCATTTCCACCTTTGCC
19	m15796	CTGAGGGCTTCGACACCTAC	TCACTCCGTCTTTTGCACAG
20	u53328	AGCCAAAGGTCTGTGGTTG	TGACATGCCTTCAGTTGAGC
21	y12065	CCTCAGGAGAATGGAATGGA	GGAAATGTCACCTTGGGCTA
22	af001601	TGATTCAGCAAATGGGATCA	AGAACAGACCCATTGTTGGC
23	nm001885	GCCCTTCTTTCCCCTTTCCAC	TCACGGGTGATGGGAATGGT

After 1 or 3 days of 0.2Gy whole body radiation, we extracted three mouse organs, including brain, lung and spleen which have been reported to have different radiosensitivity [28], to elucidate genes which might regulate intrinsic radiosensitivity. As seen in Fig 1A, hematoxylin and eosin staining at 3 days after 10Gy radiation confirmed that the brain was the most radioresistant and the spleen the most sensitive: A lot of necrotic lesions and apoptotic bodies were observed in the spleen and lung, while no alteration was found in the case of brain (Fig. 1A). Furthermore, TUNEL staining for the detection of apoptosis after whole body 0.2Gy radiation also showed similar patterns (Fig. 1A and 1B): spleen showed many TUNEL positive cells and lung showed a few. In the case of brain, no TUNEL positive cells were found after 0.2Gy radiation.

To discern the genes that govern the organ sensitivity, semi-quantitative RT-PCR using 23 primers was performed in spleen, lung and brain tissue from three each of female and male mice. Expressions of APO-1 cell surface antigen,

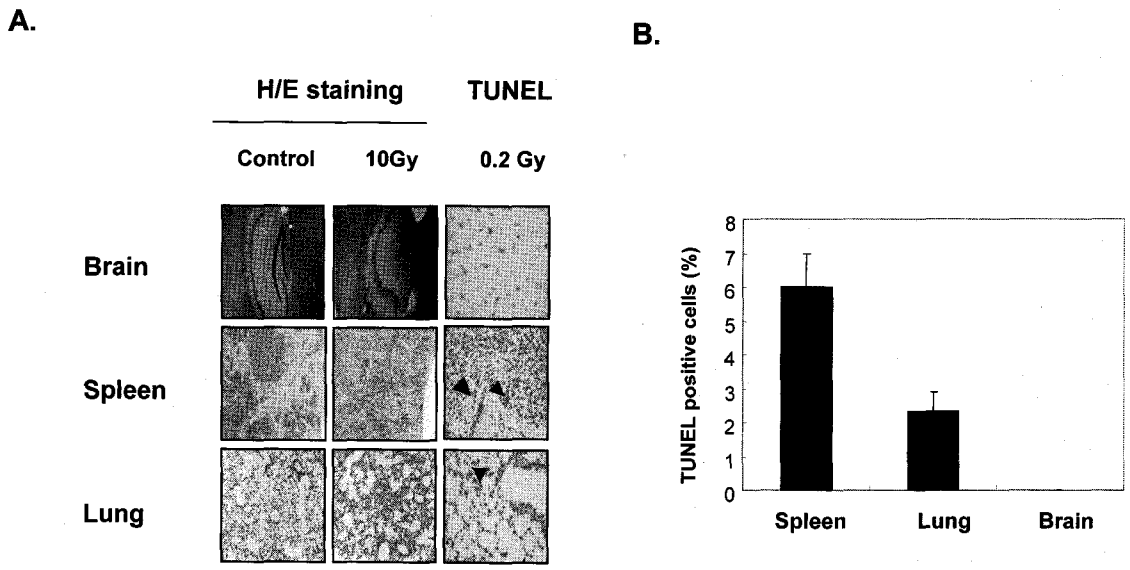

Fig. 1. Organ specific radiosensitivity.

After 3 days of 10Gy or 0.2Gy radiation, spleen, lung and brain tissue were extracted from C57BL6 mice embeddedand cut into 5 mm thick sections. Tissue blocks were fixed in 10% neutral buffered formalin, adjusted to pH7.4, and embedded in paraffin. Serial sections were prepared, and each of three adjacent sections was stained with hematoxylin and eosin or TUNEL staining. Three mice were used and similar pattern was showed. Arrow indicates TUNEL positive cells.

HSP70, nuclease sensitive element binding protein-1, cannabinoid receptor, Na, K-ATPase alpha-subunit, nucleolar protein hNop56, and paraoxonase were similar in the three organs of both female and male mice (Tables 3 and 4), indicating no gender and organ difference in the expressions of these genes of brain, lung and spleen (Table 5). Therefore, if these gene expressions were altered by radiation, they would be good candidates for a radiation response gene regardless of organs because these genes were also responded to radiation [27].

The expressions of sialyltransferase, delta7-sterol reductase, leptin receptor splice variant form 12.1, Cu/Zn superoxide dismutase (SOD) and αB crystalline were distinguished in radiosensitive spleen; expressions of sialytransferase, delta7-sterol reductase, and leptin receptor splice variant form 12.1 were very high, whereas that of αB crystalline was the lowest in the organs (Table 6). Moreover, the gene expression of sialyltransferase, Cu/ZnSOD and αB crystalline sensitively responded to 0.2 Gy radiation (data not shown), suggesting that these genes might be candidates for markers of spleen exposure to radiation. In addition, other radiosensitive organs, such as bone marrow and intestine also showed increased gene expression of sialyltransferase and Cu/ZnSOD (data not shown), suggesting that these genes might be candidates for radiosensitivity. The brain, which was the most radioresistant, showed the highest expression of APO-1 cell surface antigen and lower expression levels of Na, K-ATPase α-subunit, Cu/ZnSOD and cyclin G. In the case of lung, moderately radiosensitive organ, Na, K-ATPase α-subunit, cyclin G1 and nucleolar protein hNop56 showed higher expression, whereas the expression of delta7-sterol reductase was low. From the above results, delta7-sterol reductase appeared to be a detection marker of intrinsic radiosensitivity, because of the highest expression in spleen and the lowest expression in brain. Sialyltransferase, leptin receptor splice variant form 12.1, and Cu/ZnSOD might be specific markers for spleen,

Table 3. Expression patterns in brain, lung and spleen tissues of male mice.

Gene bank accessionno	Gene name Glutathione peroxida	Expression pattern Brain	Spleen	Lung	Brain	Spleen	Lung
D00632		++	++	+++			
Y12781	Transducin (beta) like 1 protein	+++	+++	+++			
X63717	APO-1 cell surface antigen	++	++	++++			
U02680	Protein tyrosine kinase	-	-	-			
X53586	Integrin alpha 6	++	+++	+++			
j02764	Platelet membrane glycoprotein Iib (ITGA2B)	+	++	++			
P34932	HSP70	++	++	++			
X17247	Sialyltransferase	+	+++	++			
M85234	Nuclease sensitive element binding protein-1	+++	++	++			
X54937	Cannabinoid receptor	+++	+++	+++			
l42176	DRAL	+++	+++	+++			
U32315	Syntaxin 3	-	-	-			
X04297	Na, K-ATPase alpha-subunit	+++	++	+			
af034544	Delta7-sterol reductase	+	++	++			
s69022	Myosin light chain-2	++	++	++			
U72391	Neogenin	++	+	++			
U66496	Leptin receptor splice variant form 12.1	++	++++	+++			
X02317	Cu/Zn superoxide dismutase	++	+++	++			
M15796	Cyclin protein gene	++	++	++			
U53328	Cyclin G	+++	++	+			
Y12065	Nucleolar protein hNop56	+++	+	+			
af001601	Paraoxonase (PON2)	+++	+++-	+++			
Nm001885	aB Crystallin	++	+	++			
	Beta-Actin						

Gene Expressions of each organ from 3 mice by RT-PCR was shown and the expression ratio was calculated; ++++: mean band intensity was more than 1.5, +++: mean band intensity was 1-1.5, ++: mean band intensity was 0.5-1, +: mean band density was less than 0.5

Table 4. Expression patterns in brain, lung and spleen tissues of female mice.

Gene bank accessionno	Gene name	Expression pattern					
		Brain	Spleen	Lung	Brain	Spleen	Lung
D00632	Glutathione peroxida	+++	+++	+++			
Y12781	Transducin (beta) like 1 protein	+++	+++	++			
X63717	APO-1 cell surface antigen	++	++	++++			
U02680	Protein tyrosine kinase	++	+	+			
X53586	Integrin alpha 6	+++	+++	+++			
j02764	Platelet membrane glycoprotein Iib (ITGA2B)	++	++	++			
P34932	HSP70	+++	++	++			
X17247	Sialyltransferase	+	+++	+			
M85234	Nuclease sensitive element binding protein-1	+++	+	+++			
X54937	Cannabinoid receptor	+++	+++	+++			
l42176	DRAL	+++	+++	+			
U32315	Syntaxin 3	+	+	++++			
X04297	Na, K-ATPase alpha-subunit	+++	++	++			
af034544	Delta7-sterol reductase	+	++	+			
s69022	Myosin light chain-2	+++	+++	+			
U72391	Neogenin	+	+	+			
U66496	Leptin receptor splice variant form 12.1	++++	++++	+			
X02317	Cu/Zn superoxide dismutase	++	+++	+			
M15796	Cyclin protein gene	+++	+++	+			
U53328	Cyclin G	+++	+	+			
Y12065	Nucleolar protein hNop56	+++	++	+			
af001601	Paraoxonase (PON2)	+++	+++	+++			
Nm001885	αB Crystallin	++	+	++			
	Beta-Actin						

Gene Expressions of each organ from 3 mice by RT-PCR was shown and the expression ratio was calculated; ++++: mean band intensity was more than 1.5, +++: mean band intensity was 1-1.5, ++: mean band intensity was 0.5-1, +: mean band density was less than 0.5

Table 5. Genes with similar expression pattern in spleen, lung and brain of both male and female mice.

Gene bank accession no	Gene name
x63717	APO-1 cell surface antigen
p34932	HSP70
m85234	Nuclease sensitive element binding protein-1
x54937	Cannabinoid receptor
x04297	Na, K-ATPase alpha-subunit
y12065	Nucleolar protein hNop56
af001601	Paraoxonase (PON2)

Table 6. Genes with the highest or the least expressions in spleen.

Gene bank accession no	Gene name	Male	Female
x17247	Sialytransferase	High	High
af034544	Delta7-sterol reductase	High	High
u66496	Leptin receptor splice varian form 12.1	High	High
x02317	Cu/Zn superoxide dismutase	High	High
nm001885	aB Crystallin	Low	Low

Table 7. Genes with the highest or the least expressions in lung.

Gene bank accession no	Gene name	Male	Female
d00632	Glutathione peroxidase	High	Same(all)
x63717	APO-1 cell surface antigen	High	High
x04297	Na,K-ATPase a-subunit	Low	Low
x02317	Cu/Zn superoxide dismutase	Low	Low
u53328	Cyclin G	Low	Low

Table 8. Genes with the highest or the least expressions in brain.

Gene bank accession no	Gene name	Male	Female
x04297	Na, K-ATPase a-subunit	High	High
af034544	Delta7-sterol reductase	Low	Low
u53328	Cyclin G	High	High
y12065	Nucleolar protein hNop56	High	High

APO-1 cell surface antigen for lung, and Na, K-ATPase α-subunit, cyclin G and nuclear protein hNop56 for brain.

DISCUSSION

In the present study, in order to identify genes that show organ specific expression and that regulate organ sensitivity to radiation, we analyzed 23 genes in mouse brain, lung, and spleen which have different sensitivity to radiation exposure; brain was the most resistant and spleen was the most sensitive (Fig. 1). These 23 genes represent diverse cellular functions such as antioxidation system, cell surface marker, chaperone function, and cell cycle regulation.

Our previous microarray analysis revealed 44 genes in PBL which were upregulated by 1Gy radiation when compared to unirradiated sham control PBL [27]. In the present study, we selected 23 genes out of these genes, because they were detected in at least one of 3 organs of mouse system, and performed semi-quantitative RT-PCR analysis to find genes responsible for intrinsic organ radiosensitivity (Tables 1 and 2). As shown in Table 3 and 4, expression pattern of each gene was different in 3 organs of 3 individual mice. However, expressions of APO-1 cell surface antigen, HSP70, nuclease sensitive element binding protein-1, cannabinoid receptor, Na, K-ATPase alpha-subunit, cyclin protein gene, nucleolar protein hNop56, and paraxonase (PON2) (Table 5) were not different depending on the gender and organs, indicating that these gene expressions may not be involved in gender or organ difference and may be candidates for universal markers for radiation exposure, independent of gender or organs.

The gene expression of sialyltransferase and delta7-sterol reductase which were highly expressed in the lung of male mice, and leptin receptor splice variant form 12.1 and Cu/ZnSOD were the high in spleen, while that of αB crystalline was low (Table 6). Spleen was one of the most radiosensitive organs according to TUNEL staining (Fig. 1), and these genes may play roles in controlling susceptibility to radiation exposure. Since basal gene expression of sialyltransferase was strongly detected in intestine, and the expression of genes such as sialyltransferase, Cu/ZnSOD and αB crystalline by radiation was increased in spleen or intestine, these results indicate that these genes are sensitive marker for radiation exposure (data not shown).

In the case of brain, one of radioresistant organs, Na, K-ATPase α-subunit, delta7-sterol reductase, cyclin G and nucleolar protein hNop56 were specifically either over-or under-expressed (Table 8). Since delta7-sterol reductase was the most highly expressed in spleen and the lowest in brain, this gene is the most plausible candidate for controlling radiosensitivity. Lung showed moderate radiosensitivity, compared to spleen and brain (Fig. 1), and has been known to be a target organ to induce complication such as fibrosis after radiation treatment. Therefore, further study will be required to confirm whether lung specific genes involved in radiation induce complication and radiation response.

p53 and p21 expression levels were not correlated with the organ sensitivity to radiation exposure in our system; p53 expression in spleen, lung and brain was not detected even by RT-PCR method and basal expression level of p21was similar in the three organs (data not shown). Therefore, p53 and p21 expressions could be excluded in the regulation of organ sensitivity to radiation.

In the present system, we did not consider the function of each gene, because we focused only on the genes which governed intrinsic radiation sensitivity. Of course, it is obvious that the radiation responses of organized tissues and organs are related to the radiosensitivity of individual components, functional cells, and vascular and connective tissue. The radiosensitivity of an organ is related to the effect of radiation on these individual components and the functions of these components are still in question. In addition, cytokines induced by radiation strongly influence response in an either protective or deleterious manner as well as

secondary overall response such as fibrosis and inflammation [29, 30]. Regardless of their function, however, the first goal of our present study was to identify genes that govern intrinsic organ radiosensitivity which was presented by cell death, and these genes could be used as candidate markers for radiation exposure for specific organs. We did not consider the difference of gene expression patterns in 3 different tissues in terms of how rapidly the damage is expressed in the organ. However, since these genes may modulate cellular intrinsic radiosensitvity, further functional study might shed some light on the relationship of radiosensitivity and expression profiles of genes after radiation, particularly after low dose radiation.

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