IDENTIFICATION OF GENES EXPRESSED IN LOW-DOSE-RATE γ -IRRADIATED MOUSE WHOLE BRAIN

JIN JONG BONG*, YU MI KANG*, SEUNG JIN CHOI*, DONG-KWON KIM†, KYUNG MI LEE‡, and HEE SUN KIM*

*Radiation Health Research Institute, Korea Hydro and Nuclear Power Co., Ltd.,
†Nuclear Environment Research Division, Korea Atomic Energy Research Institute,
‡Global Research Lab, BAERI Institute, Department of Biochemistry and Molecular Biology, Korea University College of Medicine

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While high-dose ionizing radiation results in long term cellular cytotoxicity, chronic low-dose (<0.2 Gy) of X- or γ-ray irradiation can be beneficial to living organisms by inducing radiation hormesis, stimulating immune function, and adaptive responses. During chronic low-dose-rate radiation (LDR) exposure, whole body of mice is exposed to radiation, however, it remains unclear if LDR causes changes in gene expression of the whole brain. Therefore, we aim to investigate expressed genes (EGs) and signaling pathways specifically regulated by LDR-irradiation (^{137}Cs, a cumulative dose of 1.7 Gy for total 100 days) in the whole brain. Using microarray analysis of whole brain RNA extracts harvested from ICR and AKR/J mice after LDR-irradiation, we discovered that two mice strains displayed distinct gene regulation patterns upon LDR-irradiation. In ICR mice, genes involved in ion transport, transition metal ion transport, and developmental cell growth were turned on while, in AKR/J mice, genes involved in sensory perception, cognition, olfactory transduction, G-protein coupled receptor pathways, inflammatory response, proteolysis, and base excision repair were found to be affected by LDR. We validated LDR-sensitive EGs by qPCR and confirmed specific upregulation of S100a7a, Olfr624, and Gm4868 genes in AKR/J mice whole brain. Therefore, our data provide the first report of genetic changes regulated by LDR in the mouse whole brain, which may affect several aspects of brain function.

Keywords: Low−dose−rate irradiation, Mouse whole brain, Radiation−sensitive expressed genes

1. INTRODUCTION

Nuclear accidents have shown that ionizing radiation has negative effects on brain development. Many studies of prenatally exposed Hiroshima and Nagasaki atomic bomb survivors have shown that exposure to ionizing radiation during gestation has harmful effects, including generalized growth retardation, small head size, and mental retardation [1,2].

In addition, ionizing radiation has been reported to affect the proliferation of brain cells. A temporary increase in proliferating cell numbers was observed in the whole rostral migratory stream of rat brain 25 days after 3 Gy exposures [3]. A recent study showed that the proliferation and apoptosis of gamma-irradiated brain cells is closely related with gene expression. Increased proliferation and decreased apoptosis of endothelial cells, as well as altered gene expression of VEGF (vascular endothelial growth factor), Tie-2 (tyrosine-protein kinase receptor), Ang-1 (angiopoietin 1), and Ang-2 (angiopoietin 2) was detected in the brains of F344 × BN rats exposed to 10 Gy gamma rays [4]. Radiation-responsive gene expression has been shown to influence apoptosis and immune responses in the brain. The differential expression of AP1 (activator protein 1), NFκB (nuclear factor kappa B), CREB (cAMP-responsive element-binding protein),

Corresponding author : Hee Sun Kim, hskimdvm@khnp.co.kr
Radiation Health Research Institute, Korea Hydro and Nuclear Power Co., Ltd., 388-1, Ssangmun-dong, Dobong-gu, Seoul 132-703, Republic of Korea

TNFα (tumor necrosis factor alpha), IL1β (interleukin 1 beta), and MCP1 (monocyte chemoattractant protein 1) induced a pro-inflammatory environment in 10 Gy-irradiated rat brains [5]. In addition, low-dose radiation can influence gene expression and immune responses; for example, the exposure of healthy tissues to low-dose radiation was shown to induce NFκB and SOD2 (superoxide dismutase 2) activity, as well as transcriptional activation of NFκB signal transduction/target molecules in the brain and gut [6]. Low-dose radiation (<200 cGy) was reported to rapidly activate the neuroimmune system, potentially causing early onset fatigue-like symptoms in mice [7].

We have investigated the radiation-sensitive EGs in various organs of ICR and AKR/J mice. In this study, we identified LDR-sensitive EGs by microarray and signaling pathway analysis in the mouse whole brain. Then, we validate LDR-sensitive EGs by qPCR and discussed their possible functions in mouse whole brain.

2. MATERIALS AND METHODS

Animals. Seven-week-old female ICR (23.9±1.17 g) and AKR/J (24±1.37 g) mice were purchased from the Shizuoka Laboratory Center (Japan) and maintained under specific pathogen-free conditions. Animals were housed in a room with a temperature of 23±2°C and a relative humidity of 50±10% with a 12-h light/dark schedule (200–300 lux; 8:00 AM to 8:00 PM). After a 1-week adaptation period, 5 mice were placed in a polycarbonate cage, and provided gamma-sterilized pellets and autoclaved water *ad libitum*. All procedures were reviewed by the Institutional Animal Care and Use Committee at the Radiation Health Research Institute (RHRI) of the Korea Hydro and Nuclear Power Co. LTD., and the mice were treated in accordance with government and RHRI guidelines for animal care.

Low-dose-rate irradiation. We reared the mice in a long-term LDR-irradiation facility equipped with a ^{137}Cs source and exposed them to 0.7 mGy h^{-1} until the cumulative dose reached 1.7 Gy to evaluate the effect of LDR-irradiation. The Sham-irradiated mice was maintained under the same conditions as the LDR-irradiation group in LDR facility but not exposed to radiation. On day 100 after irradiation, we collected the whole brains from Sham- and LDR-irradiated mice and stored them in liquid nitrogen.

Microarray analysis. Whole brain RNA from ICR and AKR/J mice on day 100 after Sham- and LDR-irradiation was collected and analyzed by whole-genome microarray analysis with a SurePrint G3 Mouse GE 8x60K Microarray (Agilent Technologies, Wilmington, DE) containing 39,430 Entrez Gene RNAs and 16,251 lincRNAs. Microarray slides were scanned with a GenePix 4000B scanner (Axon Instruments, San Diego, CA) and signal intensities were analyzed using GenePix v6.0 software. Raw data were normalized using the median normalization method. The expression level of LDR-irradiated group was normalized by that

Table 1. Quantitative Polymerase Chain Reaction Primers.

Gene accession #	Symbol	Forward (5′→3′)	Reverse (5′→3′)
NM_011044	Pck1	GGCACCTCAGTGAAGACAAA	ATGCCTTCCCAGTAAACACC
NM_009763	Bst1	TGCTCGTTATGAGCTATGGG	GTCGACACCAGCTCAAGAAA
NM_027819	Ggt6	TTCCTGCAAACTGCTGAAAC	GAGGAGATGAGGAGGAGCAT
NM_199422	S100a7a	GCAGGCAGCCATACTACATC	TTGCCCAAGATGTACAGGAA
NM_001164059	Sell	TTCAGCTTCCAGTCCAAGTG	TGACCAGTTTCCAGATGCTC
XM_111973	Smek3-ps	TGCCTTGAATATGACCCTGA	TTGGGATTGTGTTATTGGGA
NM_146958	Olfr171	ACACTTTCCCTTCTGCCACT	CGCCTCGTTCATAGTGTGTT
NM_001011865	Olfr624	TTGATTCGAATGGCTTGTTC	AGGAGGCAAGAATGAGCACT
XM_144574	Gm4868	CTTGCCTCACAAAGTCGTGT	CCATCTTTGCCCTCAAATCT
NM_009438	18S ribosomal RNA	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG

Pck1: Mus musculus phosphoenolpyruvate carboxykinase 1, cytosolic, mRNA
Bst1: Mus musculus bone marrow stromal cell antigen 1 (Bst1), mRNA
Ggt6: Mus musculus gamma-glutamyltransferase 6 (Ggt6), mRNA
S100a7a: S100 calcium binding protein A7A
Sell: Mus musculus selectin, lymphocyte (Sell), transcript variant 2, mRNA
Smek3-ps: SMEK homolog 3, suppressor of mek1 (Dictyostelium), pseudogene
Olfr171: Mus musculus olfactory receptor 171 (Olfr171), mRNA
Olfr624: Mus musculus olfactory receptor 624 (Olfr624), mRNA
Gm4868: PREDICTED: Mus musculus predicted gene 4868 (Gm4868), mRNA

of Sham-irradiated group. The expression levels represent log ratios for LDR-irradiated animals. Subsequently, EGs that were differentially expressed between ICR and AKR/J mice were filtered as follows: log ratio ≥ 1 or ≤ -1. Functional analysis of differential EGs was performed using GeneSpring GX 11.5.1 (Agilent Technologies, San Carlos, CA) and DAVID Bioinformatics tools (National Institute of Allergy and Infectious Diseases, NIH) [8].

Quantitative reverse transcription polymerase chain reaction. RNA (1 μg) was reverse transcribed using an iScript^{TM} complementary DNA synthesis kit (Bio-Rad, Hercules, CA). PCR was performed in duplicate using SYBR green (Qiagen, Hilden) and a 7500 real-time PCR machine (Applied Biosystems, Foster City, CA). The relative abundance of specific messenger RNAs was calculated by normalization to 18S ribosomal RNA using the 2-δδct method. Primers are listed in Table 1.

Statistical analyses. All statistical analyses were performed using SAS release 8.02 (SAS Institute Inc., Cary, NC). Differences in gene expression in 3 or more groups were assessed by ANOVA. Data are presented as means ±SEM.

3. RESULTS AND DISCUSSION

We investigated gene expression levels in the whole brain of ICR and AKR/J mice 100 days after Sham- and LDR-irradiation (accumulative dose of 1.7 Gy) and normalized LDR-irradiation group by Sham-irradiation group. Then, we classified the results using the following criteria: log ratio ≥ 1 or ≤ -1, expecting to find EGs and signal pathways. Signal pathways for LDR-sensitive EGs were analyzed using DAVID Bioinformatics Resources v6.7. In ICR mice, 252 EGs (upregulated: 43 genes, downregulated: 209 genes) were classified and iron ion transport, transition metal ion transport, and developmental cell growth signaling pathways were analyzed (Table 2). In AKR/J mice, 380 EGs (upregulated: 212 genes, downregulated: 168 genes) were classified and sensory perception of smell, sensory perception of chemical stimulus, G protein coupled receptor protein signaling pathway, sensory perception, neurological system process, cognition, cell surface receptor linked signal transduction, positive regulation of response to stimulus, positive regulation of immune system process, acute inflammatory response, inflammatory response, defense response, and proteolysis signaling pathway were analyzed (Table 2). We classified LDR-sensitive EGs, and then were validated by qPCR analysis. Thus, gene expression of

Table 2. Signal Pathway in Whole Brain of ICR and AKR/J Mice 100 Days after Low-Dose-Rate Irradiation.

	Term†	Number of genes‡				P value
		Up	%	Down	%	
ICR mice	Iron ion transport	2	4.7	0	0	0.002
	Transition metal ion transport	2	4.7	0	0	0.004
	Developmental cell growth	0	0.0	2	1.0	0.031
	Total number	43		209		
AKR/J mice	Sensory perception of smell	11	5.2	0	0.0	0.002
	Sensory perception of chemical stimulus	11	5.2	0	0.0	0.003
	G-protein coupled receptor protein signaling pathway	14	6.6	0	0.0	0.003
	Sensory perception	11	5.2	0	0.0	0.009
	Neurological system process	12	5.7	0	0.0	0.011
	Cognition	11	5.2	0	0.0	0.013
	Cell surface receptor linked signal transduction	15	7.1	0	0.0	0.015
	Positive regulation of response to stimulus	4	1.9	0	0.0	0.020
	Positive regulation of immune system process	4	1.9	0	0.0	0.026
	Acute inflammatory response	3	1.4	0	0.0	0.026
	Inflammatory response	4	1.9	0	0.0	0.032
	Defense response	5	2.4	0	0.0	0.049
	Proteolysis	0	0.0	12	7.1	0.001
	Base excision repair	3	1.4	0.0	0.0	0.017
	Olfactory transduction	10	4.7	0.0	0.0	0.041
	Total number	212		168		

† Signaling pathways were analyzed with DAVID Bioinformatics Resources v6.7

‡ Differential expressed genes were classified as log ratio ≥ 1 or ≤ -1

Up (up-regulated genes), Down (down-regulated genes)

Table 3. Profiles of LDR-Sensitive Expressed Genes in Mice Whole Brain.

Function	Gene symbol	Microarray†		qPCR‡			Validation
		ICR	AKR/J	ICR	AKR/J	P value	(y or ?)
Gluconeogenesis	Pck1	1.2	-2.9	3.5 ± 1.7	18.6 ± 8.7	0.015	?
Immune response and inflammation	Bst1	1.5	0.8	1.8 ± 0.6	6.0 ± 2.2	0.012	?
	Ggt6	-1.0	0.0	4.1 ± 2.2	9.1 ± 3.2	0.039	?
	S100a7a	-1.2	7.3	4.1 ± 1.2	10.8 ± 3.2	0.008	y
	Sell	-0.1	-7.0	2.3 ± 1.0	11.5 ± 2.9	0.001	?
	Smek3-ps	-5.4	6.8	19.8 ± 9.3	3.8 ± 1.2	0.015	?
Neurotransmitter	Olfr171	-0.1	-7.2	4.1 ± 1.6	21.5 ± 5.3	0.001	?
	Olfr624	-0.1	6.6	4.0 ± 0.7	22.6 ± 5.3	<0.001	y
Unknown	Gm4868	0.1	7.7	2.9 ± 1.5	7.8 ± 2.1	0.009	y

† The expression value of LDR-irradiated group was normalized to that of sham-irradiated group.

‡ 18S ribosomal RNA was used as a housekeeping reference gene for quantitative polymerase chain reaction (qPCR) analysis. Each value represents the mean ± standard error. The 2-tailed Student's t-test with unequal variance was used for statistical analysis. $p < 0.05$ was considered statistically significant. Experiments were carried out in duplicate.

? : Microarray data was not matched with qPCR analysis

S100a7a, Olfr624, and Gm4868 was upregulated in AKR/J mice (Table 3). Therefore, we identified LDR-sensitive EGs and signal pathways in the mice whole brain.

Ionizing radiation has a harmful effect on the development of the brain. Studies on individuals exposed prenatally to ionizing radiation showed that the neurons of their central nervous systems are not self-renewing, and thus, neuronal loss cannot be repaired through repopulation [2]. Subsequently, ionizing radiation was shown to affect the proliferation, differentiation, and apoptosis of brain cells. Verheyde and Benotmane reviewed that exposure of the developing brain to ionizing radiation could lead to disturbances in cell proliferation, migration, differentiation, cell death, the generation of morphological abnormalities, as well as a disturbance in regional brain formation [9]. Previous study showed that carcinogenic cells have been removed by activated apoptosis and immune mechanisms, contributing to decreased thymic lymphoma and elongated life span in low-dose-rate irradiated AKR/J mice [12]. According to reference, AKR/J mice has murine leukemia virus, which was presented in brain, spleen, and thymus [10]. Then, this study used ICR mice as a control, which were less sensitive to LDR-irradiation. Thus, we investigated the effects of LDR-irradiation (0.7 mGy h^{-1}, a cumulative dose of 1.7 Gy) on the whole brain of ICR and AKR/J mice by a microarray and gene expression profiling study.

Whole brain of LDR-irradiated ICR and AKR/J mice showed a different patterns of gene expression and signal pathway. Interestingly, transcriptional changes were more detected in the whole brain of AKR/J mice compared to that of ICR mice 100 days after receiving LDR-irradiation (0.7 mGy h^{-1}, a cumulative dose of 1.7 Gy). Moreover, signaling pathway analysis showed that in ICR mice, LDR-sensitive EGs participated in ion transport, transition metal ion transport, and developmental cell growth, whereas LDR-sensitive EGs participated in sensory perception, G protein coupled receptor protein signaling pathway, neurological system process, cognition, cell surface receptor linked signal transduction, stimulus response, immune system process, inflammatory response, defense response, proteolysis, base excision repair, and olfactory transduction in AKR/J mice. In accordance with signal pathway analysis, recent studies reported that LDR-irradiation stimulated immune response- and DNA repair-related genes expression [11,12]. Together with these results, LDR-irradiation may contribute to immune response and DNA repair pathway in AKR/J mice. Then, LDR-sensitive EGs were categorized in gluconeogenesis (Pck1), immune response and inflammation (Bst1, Ggt6, S100a7a, Sell, Smek3-ps), neurotransmitters (Olfr171, Olfr624), and unknown functions (Gm4868). Thus, we validated LDR-sensitive EGs by qPCR analysis. Therefore, we found that gene expression of S100a7a, Olfr624, and Gm4868 was upregulated in AKR/J mice.

S100 proteins are well-known as calcium-binding proteins that control basic biological processes. Eckert reviewed that S100A7 played an role on a transglutaminase substrate/cornified envelope precursor, signal transduction protein, chemokine, and antibacterial protein in normal epidermis [13]. Also, Lee reported that S100A7 was highly expressed in epidermal hyperproliferative disorders [14]. Furthermore, S100A7 has been shown to be overexpressed during inflammation responses [15-18]. Recent studies reported that S100A7 was involved in brain disease. For example, Zhang reported that S100A7 expression was closely associated with squamous cell carcinoma metastasis to brain [19].

In addition, Qin reported that upregulation of S100A7 expression might selectively promote alpha-secretase activity in the brain of Alzheimer's disease [20]. Furthermore, It was reported that S100A7 affected immune system in the bacterial- or virus-infected brain. For example, Jansen reported that S100A7 might modulate the innate immune system in the brain after bacterial or viral stimulation [21]. Together with these reports, we suggest that LDR-irradiation may contribute to the basic biological processes, immune response and inflammation via the upregulation of S100a7a in AKR/J mice whole brain. We also found that gene expression of Olfr624 and Gm4868 was upregulated in AKR/J mice whole brain. Olfactory receptors interact with odorant molecules in the nose, to initiate a neuronal response that triggers the perception of a smell [22]. Recent studies reported that olfactory receptor family affected brain disease and injury. For example, gene expression of olfactory receptor family was downregulated in the cerebral cortex of Parkinson disease patients [23]. Zhao reported that olfactory receptor family was novel therapeutic targets for traumatic brain injury [24]. Together with these reports, we suggest that LDR-irradiation may affect perception, brain disease, and brain injury via the upregulation of Olfr624 in AKR/J mice. However, the functions of Gm4868 is unknown.

4. CONCLUSION

We identified the upregulation of S100a7a, Olfr624, and Gm4868 in the whole brain of AKR/J mice after LDR-irradiation. Then, LDR-sensitive EGs were involved in the basic biological processes, immune response, inflammation, brain disease, and brain injury in the AKR/J mice. However, the functions of EGs after LDR-irradiation require further study and validation. We expect that LDR-sensitive EGs and pathways will be useful genetic markers for diagnosing cancers and for enhancing the efficacy of radiotherapy.

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