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Integrative Analysis for the Roles of lncRNAs in the Immune Responses of Mouse PBMC Exposed to Low-Dose Ionizing Radiation

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Abstract

It is well accepted that low-dose ionizing radiation (LDIR) modulates a variety of immune responses that have exhibited the properties of immune hormesis. Alterations in messenger RNA (mRNA) and long noncoding RNA (lncRNA) expression were to crucially underlie these LDIR responses. However, lncRNAs in LDIR-induced immune responses have been rarely reported, and its functions and molecular mechanisms have not yet been characterized. Here, we used microarray profiling to determine lncRNA in BALB/c mice exposed to single (0.5 Gy×1) and chronic (0.05 Gy×10) low-dose γ-rays radiation (Co^{60}). We observed that a total of 8274 lncRNAs and 7240 mRNAs were altered in single LDIR, while 2077 lncRNAs and 796 mRNAs in chronic LDIR. The biological functions of these upregulated mRNAs in both 2 groups using Gene Ontology functional and pathway enrichment analysis were significantly enriched in immune processes and immune signaling pathways. Subsequently, we screened out the lncRNAs involved in regulating these immune signaling pathways and examined their potential functions by lncRNAs-mRNAs coexpression networks. This is the first study to comprehensively identify lncRNAs in single and chronic LDIR responses and to demonstrate the involvement of different lncRNA expression patterns in LDIR-induced immune signaling pathways. Further systematic research on these lncRNAs will provide new insights into our understanding of LDIR-modulated immune hormesis responses.

Keywords

low-dose irradiation, immune response, lncRNAs, microarray, expression profiles

Introduction

All human beings are inevitably exposed to low doses of natural and anthropogenic ionizing radiation (IR) in their daily life. Accumulating data suggest that the biological responses to high and low doses of radiation are qualitatively different. It is widely accepted that IR at high doses can damage normal tissue and lead to organ dysfunction and death in severe cases. However, the biological effects of low-dose ionizing radiation (LDIR) exposures, such as adaptive response and hormetic effect, are still inconsistent and inconclusive.[1]

The immune system, one of the most important defenses against environmental insults and stresses, is strongly affected by IR.[2] Many experimental studies showed that high-dose radiation suppresses the immune system, while low-dose radiation may stimulate it.[3] Low-dose ionizing radiation can not only regulate a variety of immune response processes but can reveal the properties of immune hormesis.[4] Over the past several decades, increasing studies show the hormetic effect of LDIR on the immune system is beneficial for human health, including enhancing immune functions, delaying cancer development, inhibiting the aging process, and so on.[4] Although the

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underlying molecular mechanism is not fully understood yet, LDIR has been used clinically for alleviating autoimmune diseases by controlling overactive autoimmune reactions and inhibit malignant tumors growth, metastasis, and occurrence by enhancing the immune response.[5-7] And the therapeutic potential of low-dose radiation has been systematically investigated on different animal models of immune-related diseases.[7-9] Hence, it is worthwhile to further research LDIR-modulated immune response in animal model because of its enormous clinical potential.

Nevertheless, the understanding on the underlying mechanisms of LDIR-induced immune hormesis is still fragmented and incomplete. The researches on gene expression alterations helped to understand the molecular mechanisms of LDIR-induced immune hormetic effect.[10] Accumulating evidence indicates that long noncoding RNAs (lncRNAs) modulate transcription or posttranscriptional processes, participate in a wide variety of important biological events such as chromosome dosage compensation, genomic imprinting, and functional protein trafficking,[11] as well as closely related to diverse human diseases, including tumorigenesis and autoimmune disease.[12,13] Various studies have revealed that lncRNAs may play important roles in fighting against pathogens and maintaining normal health and homeostasis by influencing the transcriptional programs of immune cells.[14] Therefore, analyzing the expression profiles of lncRNAs may provide new insights into our understanding of the molecular mechanism of the LDIR-induced immune response and discover some lncRNAs as potential valuable candidate biomarkers for radiation biodosimetry. However, previous studies focused on the changes in gene expression following direct low-dose radiation.[15,16] For instance, lncRNA PARTICLE modulated the expression of tumor suppressor MAT2A by regulating locus-specific methylation in response to LDIR and was as a candidate biomarker in patient plasma post-radiotherapy.[17] Microarray analysis allows us to comprehensively understand the radiation-induced responses by identify gene expression.[18] For example, a research demonstrated that lncRNAs could serve as biomarkers for radiation biodosimetry and filter out 2 lncRNAs served as IR biomarkers by analyzing the gene expression profiles in a mouse model after whole-body exposed to single acute irradiation.[19] However, there is still a lack of systematic research and analysis on the expression profiles of lncRNAs in vivo and in vitro experiments response to LDIR. Until now, a recent research on the lncRNA expression profiles in either BALB/c or SPRET/EiJ mice exposed to LDIR exposure (10 cGy) revealed the vast majority of differentially expressed lncRNAs had a significantly correlated with at least 1 LDIR responsive messenger RNA (mRNA).[20] Gao et al determined the expression profile of lncRNAs in mouse thymocytes using integrative analysis and deduced the potential functions of lncRNAs in response to LDIR and high-dose irradiation (HDIR).[21] Although some reports have considered the roles of lncRNAs in some LDIR-induced biological effects, the relationship between the immune response and the altered expression of lncRNAs after LDIR exposure has not been addressed.

Therefore, this prompted us to carry out the comprehensive study on the expression profiles of lncRNAs in a mouse model exposed to long-term and single LDIR in order to screen out lncRNAs in immune response and help to understand the molecular mechanisms of the immune response to LDIR. In this study, we used DNA microarray and bioinformatics to investigate the profiles of lncRNAs and mRNAs from peripheral blood mononuclear cell (PBMC) of BALB/c mice at 24 hours after γ-radiation Co^{60} with 0.05 Gy for 10 times and 0.5 Gy for 1 time. The results showed the massive genes were differentially expressed after both 0.05 Gy×10 and 0.5 Gy×1 radiation in comparison to sham-irradiated (0 Gy) mice. The immune processes and immune signaling pathways were significantly triggered in the mice exposed to both single and chronic LDIR by using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. Of the total 1234 same differentially expressed lncRNAs between the mice exposed 0.5 Gy×1 and 0.05 Gy×10 irradiation, 1041 had a significantly correlated expression pattern with at least 1 differentially expressed mRNAs enriched in the immune signaling pathways triggered by 0.05 Gy×10 irradiation, while 1104 in 0.5 Gy×1 irradiation. This is the first study to comprehensively identify lncRNAs in single and chronic LDIR responses. Our results indicated that lncRNAs may exert a crucial role in the regulation of mRNA expression in immune response induced by LDIR. Further research on some lncRNAs help to understand the biological and molecular mechanisms in the immune responses of the LDIR and may result in novel therapeutic approaches.

Materials and Methods

Mouse Irradiation and Isolation of Their PBMC for RNA Extraction

BALB/c male mice (6-8 weeks old, 18-22 g weight) were purchased from Fengtai Animal Center, Beijing, China, and all of the mice were housed at the Animal Laboratory Division, Beijing Key Laboratory for Radiobiology, Beijing Institute of Radiation Medicine, Academy of Military Medical Sciences (AMMS), Beijing, China. After mice were raised for a week, the 9 mice were randomly equally divided into 3 groups: the control group (Con), single low-dose (0.5 Gy×1 dose) test group, and chronic low-dose (0.05 Gy per dose ×10 doses) test group. The mice were placed in individual containers and were whole-body irradiated using γ-radiation (Co^{60}) at the AMMS Radiation Laboratory. Low-dose test group was exposed to radiation once per week and the last exposure in the low-dose test group was completed on the same day as that in the high-dose test group. Peripheral blood lymphocytes of mice were collected for gene expression profile at 24 hours after irradiation. Peripheral blood lymphocytes were isolated by centrifugation at a speed of 500*g* for 25 minutes using lymphocyte separation medium (Hao Yang Biotechnology Company, Tianjin, China). Total RNA from the mouse PBMC was extracted using TRIzol reagent (Sigma, St. Louis, MO)

Table 1. Primer Sequences Used for Real-Time-qPCR.

Biotype	Gene Name	Forward Sequence (5′ → 3′)	Reverse Sequence (5′ → 3′)	Product Length (bp)
mRNA	NM_008361	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG	116
	NM_008392	AATGAAACCTTGGGTCTTATGCC	TGCCCATGACTTATCCAGACAG	170
	NM_001159394	GCTCCGACTCCTCCGATTTC	GAGTTCTTCACGCGAACACC	166
	NM_001112715	GACAGCCACCACAATCAACAT	CCCAGGCAGCAGAAGTTCAT	96
	NM_011436	AGCAGGAGGGAGTCGAGAC	GTTCCTAGCCGGAGATCGC	170
LncRNA	NONMMUT034142	TGCAATGAATGTCCACCCAC	GGCTATGTTCAGATGCGCTC	129
	NONMMUT004165	GCAGTAGGCAAAGGAACGATGTA	CACAAGGGACACGAGGTGAAAT	193
	NONMMUT004166	GTGGGAGCTAGATGCAGGAGAAG	CCAGGAAGGATGGGAAATGTGTA	170
	NONMMUT013955	AAACACCAGAAGAGGGCACCAG	GGCAAGAGCCAAACACTACC	180
	NONMMUT062665	CCCTTCCCTATTCTGAGTTTC	CATTACAGGTGGTTGTGAGCC	196
	NONMMUT059455	ATTCCTCAGTTCCTCCTTCTCC	TGCCCTTCCAGATACTGCTTC	293
	NONMMUT000877	GTCAGAAGAGGGCTTTGGATCGGT	GGCTAAGATGGCTCAGTGGTAAAC	110
	NONMMUT007567	GCTAGGTGGTGGGTGAGTCAAT	CCAAGAGGTTACAGGTTAGGGTC	247
	NONMMUT013385	CTGACAACAGCTCGAATGACC	ACTGACAGAGTGTCCCAGACCA	238
	NONMMUT051827	CCTTTCACAGTTAGAGCCACCA	GTACAGCCTCCTTCAGACCTTA	229

Abbreviations: lncRNA, long noncoding RNA; mRNA, messenger RNA.

according to the manufacturer's instructions after the cell pellet was washed by phosphate-buffered saline. All of the animal care and study protocols were in accordance with the guidelines of the Animal Laboratory Division, Beijing Key Laboratory for Radiobiology, Beijing Institute of Radiation Medicine, AMMS.

Bone Marrow Micronucleated Polychromatic Erythrocytes Frequency

Mice were euthanized by cervical dislocation after peripheral blood lymphocytes of mice were collected. Micronucleus (MN) assays in bone marrow PCEs were conducted using standardized procedures.[22] Isolation and staining of bone marrow cells from femoral bones of each mouse were extracted according to a previously described method.[23] Stained cells were analyzed by using an Olympus BX61 microscope (model: BX61TRF; Olympus Corporation, Tokyo, Japan) for determination of micronucleated polychromatic erythrocyte (MN-PCE) frequency. One thousand PCEs were counted to quantify MN frequency on each slide (2000 total PCE per animal). Micronucleated polychromatic erythrocyte frequencies (MN-PCE‰) were calculated as (total number of MN scored/1000 PCEs) × 1000.

Expression Profile by Microarray

Quality and quantity of total RNA from peripheral blood lymphocytes were assessed using Agilent Bioanalyzer and Nanodrop 2000. GeneChip Mouse Transcriptome Assay 1.0 was used according to the manufacturer's protocol (arrays contained 22 414 mRNAs, 36 121 lncRNAs, and 7422 other noncoding RNAs). Slides were washed and scanned on a GeneChip Scanner 3000 7G (Affymetrix, Santa Clara, CA). All processes were done by Premedical Laboratories Co, Ltd (Z-Park, Beijing, China). Data were analyzed with Affymetrix GeneChip Operating Software (Affymetrix, Santa Clara, CA). Raw signal intensities for each probe were normalized to the 75th percentile of its array. Differential expression genes between sham and irradiated were identified by the unpaired Student t test with ≥ 1.1 change and a P value $\leq .05$.

Complementary DNA Synthesis and Real-Time Quantitative Polymerase Chain Reaction

For total RNA from each peripheral blood lymphocytes, the remaining RNA from gene expression microarrays was reverse transcribed to complementary DNA using PrimeScriptRT reagent kit with gDNA Eraser (TaKaRa, Kyoto, Japan). Real-time quantitative polymerase chain reaction (RT-qPCR) was performed to quantitate the expression level of different expression genes on MyiQTM2 2-color real-time PCR detection (Bio-Rad, Hercules, CA) by using the ITaq Universal SYBR Green Supermix (Bio-Rad). Polymerase chain reactions were carried out in a 25-μL volume in triplicate duplicates and were normalized against β-actin. Amplification steps were as follows: a pre-incubation step at 95°C for 15 minutes followed by denaturation at 95°C 10 seconds, annealing and extension 55°C for 30 seconds for 35 cycles. The relative expression of evaluated genes was calculated by $2^{-\Delta\Delta Ct}$ method. Primer sequences used in studies are given in Table 1.

Functional and Pathway Enrichment Analysis

On the basis of genes biological processes of GO terms, different expression genes were classified by using Database for Annotation, Visualization and Integrated Discovery (DAVID) tool.[24] The significance of GO term enrichment in the differentially expressed mRNA list was denoted by P value with the delimited point as P value $<.05$. Functional pathways for the differentially expressed mRNAs were also enriched based on KEGG database.[25] The biological pathways for which a

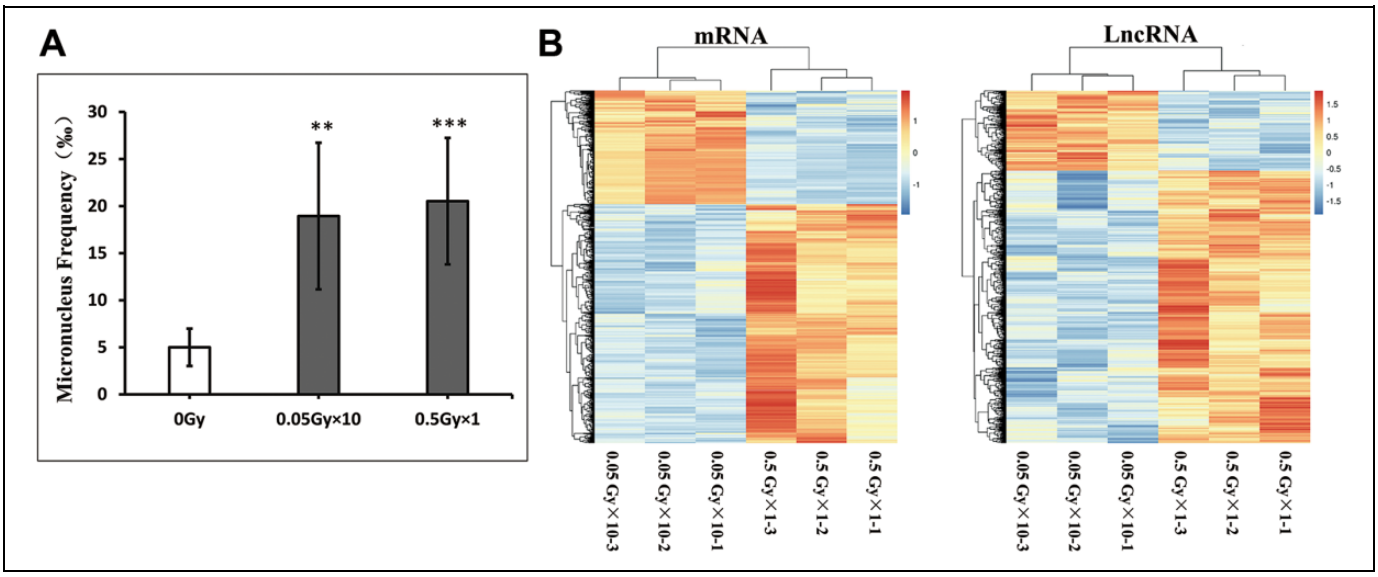

Figure 1. Effect of the irradiation with 0.05 Gy×10 and 0.5 Gy×1 doses on micronuclei frequency in bone marrow polychromatic erythrocyte (MN-PCE) and the expression profiles of lncRNAs and mRNAs in peripheral blood lymphocyte (PBMC). A, It shows MN-PCE frequencies in bone marrow cells at 24-hour postirradiation. Data were presented as mean ± standard deviation. Value of significance, $^{**}P < .01$, $^{***}P < .001$. *P* values were calculated by the unpaired Student *t* test. B, The heat maps show the hierarchical clustering of altered lncRNAs or mRNAs between the 2 groups. Red represents upregulation, and blue represent downregulation. lncRNA indicates long noncoding RNA; MN-PCE, micronucleated polychromatic erythrocytes; mRNA, messenger RNA.

significant enrichment of differentially expressed mRNAs existed ($P < .05$ was considered statistically significant).

Construction of the Co-expression Network

For each group, the average expression values of the 3 biological replicates were first calculated for each mRNA and each lncRNA and *P* values were obtained by the unpaired Student *t* test. To identify interactions among the differentially expressed lncRNAs and mRNAs (≥1.1 fold, $P < .05$), we constructed a coexpression network based on a correlation analysis of the differentially expressed lncRNAs and mRNAs.[26,27] For each potential mRNA and lncRNA pair, a correlation coefficient was calculated between the mRNAs enriched in immune signaling pathways and differently expressed lncRNAs values using R statistical analysis (Supplement Tables S1 and S2). The resulting Pearson correlation matrix was transformed into an adjacency matrix. The nodes of coexpression network correspond to gene expressions, and edges between genes are determined by the correlations between gene expressions. Pearson correlation coefficient ≥0.90 was considered statistically significant. The coexpression network was drawn with the Cytoscape (v2.8.1) software (University of Toronto, Ontario, Canada).

Results

Micronucleated Polychromatic Erythrocyte and Differentially Expressed mRNA and LncRNA

As shown in Figure 1A, compared with the MN yield in the control group (5.00%), the MN yields in the chronic low-dose irradiation (0.05 Gy ×10; LT10) and the single low-dose irradiation (0.5 Gy×1; LT1) were both significantly increased to 18.94% and 20.52%, respectively. These results indicated significant irradiation responses had occurred in this mouse model. As such, this mouse model could be applied in microarray profiling. Peripheral blood lymphocytes of LT1 and LT10 mice were collected for gene expression profile at 24 hours after irradiation exposures. Sham-irradiated (0 Gy) animals served as controls. Figure 1B shows significant differences in lncRNA and coding RNA expression in PBMC of the LT1 and LT10 mice. And then the different expression genes of LT1 and LT10 group compared with sham-irradiation group were then annotated and filtered according to fold change (≥1.1 or ≤−1.1) and gene type, respectively. The heat map illustrated the significant differentially expressed protein-coding genes and lncRNAs in both LT1 and LT10 in comparison to the sham-irradiation group (Figure 2A). It was observed that 796 mRNAs and 2077 lncRNAs were detected as differentially expressed for LT10 group, while 7240 mRNAs and 8274 lncRNAs were observed as differentially expressed for LT1 group (Figure 2B). The number and variation amplitude of differentially expressed genes was significantly greater in LT1 group in comparison with LT10 group (Figure 2B; Table 2). The expressions of 524 mRNAs and 1234 lncRNAs were changed in the both irradiation groups compared to sham-irradiated group (Figure 2B). Among them, the top 25 significantly altered genes are listed in Table 2. Significant differences in a well-characterized lncRNA (uc029rbd.1) expression were found in LT10 group and LT1 group (Table 2).

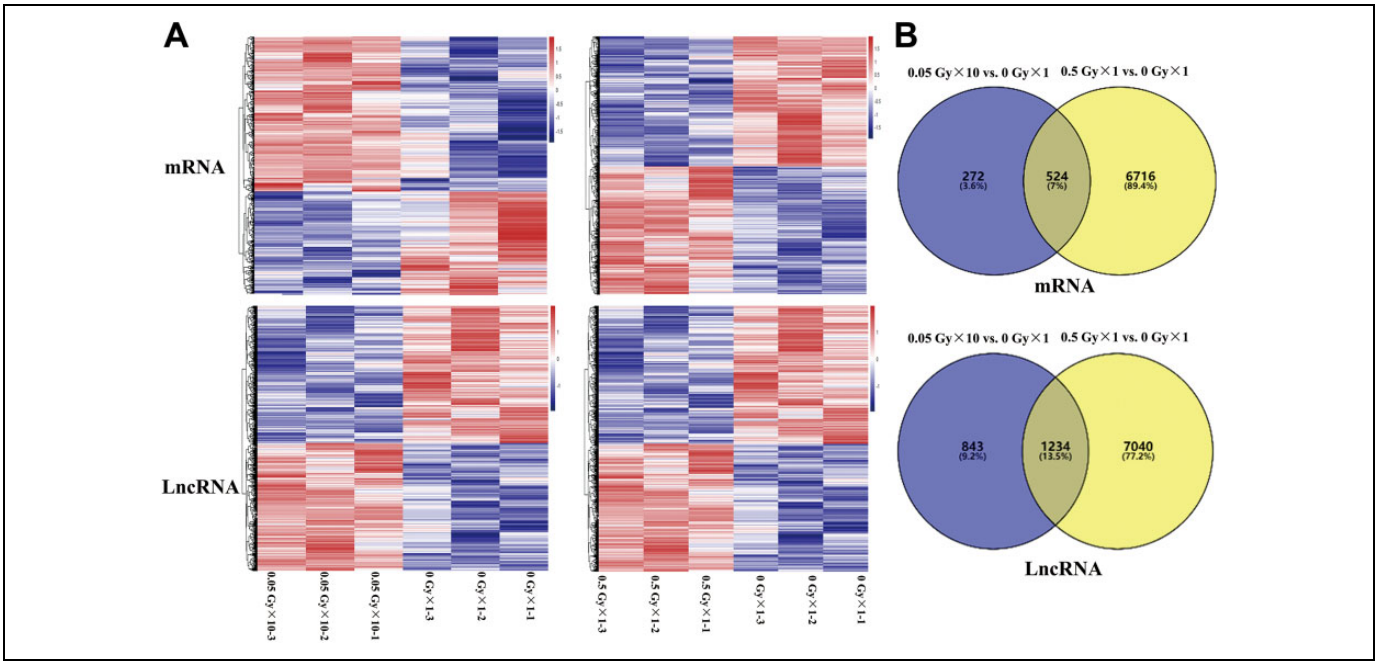

Figure 2. Expression profile changes in lncRNAs and mRNAs after exposure to the LDIR. A, The heat map and hierarchical clustering of differentially expressed mRNAs and lncRNAs response to 0.05 Gy×10 and 0.5 Gy×1 irradiation in comparison with the sham-irradiated control group, respectively. B, The Venn diagrams were used to show differentially changed mRNAs and lncRNAs in the 0.05 Gy×10 irradiated and 0.5 Gy×1 irradiated groups. LDIR indicates low-dose ionizing radiation; lncRNA, long noncoding RNA; mRNA, messenger RNA.

Validation of Differentially Expressed Genes by RT-qPCR

To verify the microarray results, we used RT-qPCR to randomly analyze the expression levels for 15 differently expressed genes, including 5 mRNAs and 10 lncRNAs, obtained using GeneChip Mouse Transcriptome Assay 1.0 (Figure 3). The same RNA samples were used for RT-qPCR as were previously analyzed in the microarray experiment. The RT-qPCR results showed that randomly selected genes from Table 2 were upregulated in both LT1 and LT10 groups compared to sham-irradiated group, which were consistent with those from microarray data (Figure 3).

Gene Ontology Functional and Pathway Enrichment Analysis

To analyze our microarray data based on groups of functionally related genes instead of individual genes, we performed functional enrichment analysis on differently expressed gene sets to demonstrate their relationships by GO and KEGG pathway analysis using DAVID tool. The upregulated GO biological processes are shown in Figure 4. There were 419 and 420 corresponding upregulated genes involved in GO biological processes of the LT10 and LT1 groups, respectively (Figure 4). Gene Ontology biological processes displayed that the mRNAs upregulated in response to both single and chronic LDIR were markedly enriched in immune response, inflammatory response, immune system process, and the activation of immune cells. The results of KEGG signaling pathway analysis in top 10 upregulated pathways showed that the upregulated genes were mainly enriched in immune signaling pathways including Toll-like receptor, T-cell receptor, NOD-like receptor, and B-cell receptor signaling pathway in both LT10 and LT1 groups, which were consistent with GO analysis results (Figure 5). The GO and KEGG analysis results indicated that immune signaling pathways can indeed be activated by the LDIR, either chronic or single irradiation.

Long Noncoding RNAs-mRNAs Coexpression Network

In order to screen out lncRNAs related to LDIR-induced immune signaling pathways, we first extracted the 15 same mRNAs enriched in the common immune pathways (Toll-like receptor, T-cell receptor, NOD-like receptor, and B-cell receptor signaling pathway) and the 1234 same differently expressed lncRNAs between LT10 and LT1 group and calculated the relationship between the same differentially expressed lncRNAs and 15 same mRNAs using Pearson correlation coefficient (Supplemental Tables S1 and S2). The relationship network nodes represent genes, and connections between the 2 nodes represent interactions between genes. As shown in Supplemental Table S1, the whole coexpression network consisted of 1056 network nodes and 3365 connections among the 15 mRNAs and 1041 differentially expressed lncRNAs in LT10 group with Pearson correlation coefficients ≥ 0.9 and P value $<.05$, while 1104 network nodes and 7050 connections among the 15 differentially expressed mRNAs and 1089 differentially expressed lncRNAs in LT1 group (Supplemental Table S2). Moreover, our data showed that 1 mRNA may correlate with

Table 2. Top 25 Same Differentially Expressed mRNAs and LncRNAs in Microarray Analysis of 0.05 Gy×10 and 0.5 Gy×1 Compared to 0 Gy Group.

mRNA								LncRNA							
	0.05 Gy×10		0.5 Gy×1						0.05 Gy×10		0.5 Gy×1				
Gene ID	*P* Value	Fold Change	*P* Value	Fold Change	Regulation	Chr	Strand	Gene ID	*P* Value	Fold Change	*P* Value	Fold Change	Regulation	Chr	Strand
NM_015811	.002	2.053	.002	2.000	up	chr1	−	uc029rbd.1	.015	3.323	.005	4.567	up	chr10	+
NM_153511	.021	1.865	.000	7.221	up	chr2	+	NONMMUT004165	.003	2.724	.000	4.769	up	chr1	+
ENSMUST00000103364	.000	1.855	.000	2.718	up	chr6	−	NONMMUT013955	.001	2.533	.000	4.336	up	chr12	−
NM_008361	.015	1.732	.000	4.988	up	chr2	−	NONMMUT004166	.006	2.531	.000	4.498	up	chr1	+
NM_009909	.041	1.555	.000	4.264	up	chr1	+	NONMMUT015969	.000	2.136	.000	3.057	up	chr12	+
ENSMUST00000103542	.013	1.553	.012	1.561	up	chr12	−	NONMMUT063950	.001	2.106	.000	2.615	up	chr7	−
ENSMUST00000103313	.007	1.549	.008	1.536	up	chr6	−	NONMMUT026195	.000	2.053	.000	2.836	up	chr16	−
NM_007569	.009	1.475	.000	2.138	up	chr10	+	KnowTID_00007598	.003	2.013	.001	2.578	up	chr9	+
NM_001159394	.004	1.462	.000	2.515	up	chr16	−	NONMMUT007567	.004	1.983	.000	2.862	up	chr10	−
ENSMUST00000054760	.027	1.455	.004	1.747	up	chr10	−	NONMMUT034142	.008	1.951	.000	8.164	up	chr19	−
NM_011279	.029	1.432	.012	1.547	up	chr9	−	NONMMUT043227	.000	1.896	.000	2.636	up	chr3	+
NM_153175	.005	1.423	.000	1.901	up	chr6	−	NONMMUT023175	.002	1.817	.000	2.560	up	chr15	−
ENSMUST00000112062	.001	1.409	.000	2.019	up	chr2	−	NONMMUT000877	.007	1.741	.000	2.870	up	chr1	−
NM_008392	.030	1.409	.000	3.993	up	chr14	+	NONMMUT031855	.007	1.732	.000	2.609	up	chr18	+
ENSMUST00000084055	.026	1.408	.018	1.452	up	chr8	+	NONMMUT005859	.004	1.698	.000	3.604	up	chr10	−
uc008czh.1	.024	1.378	.001	1.824	up	chr17	−	NONMMUT051827	.003	1.672	.000	2.632	up	chr5	+
NM_174960	.006	1.372	.000	1.659	up	chr6	+	NONMMUT051826	.022	1.652	.001	2.408	up	chr5	+
ENSMUST00000103502	.027	1.370	.028	1.367	up	chr12	−	NONMMUT013385	.005	1.645	.000	2.646	up	chr12	+
NM_029723	.007	1.364	.004	1.417	up	chr2	+	NR_045640	.008	1.644	.000	2.993	up	chr9	−
ENSMUST00000178768	.011	1.364	.000	1.765	up	chr14	+	NONMMUT031639	.011	1.622	.000	2.641	up	chr18	+
NM_001112715	.042	1.362	.000	2.206	up	chr7	+	NONMMUT059455	.014	1.612	.000	2.998	up	chr6	−
NM_001079694	.009	1.355	.000	1.940	up	chr12	+	NONMMUT070390	.007	1.606	.000	2.963	up	chr9	−
ENSMUST00000103643	.013	1.353	.000	1.779	up	chr14	+	NONMMUT001680	.001	1.600	.000	2.282	up	chr1	−
ENSMUST00000099948	.004	1.348	.000	1.854	up	chr2	+	NONMMUT042990	.000	1.575	.000	2.484	up	chr3	−
ENSMUST00000178325	.004	1.348	.000	1.854	up	chr2	+	NONMMUT001267	.009	1.570	.000	2.157	up	chr1	−

Abbreviations: lncRNA, long noncoding RNA; mRNA, messenger RNA.

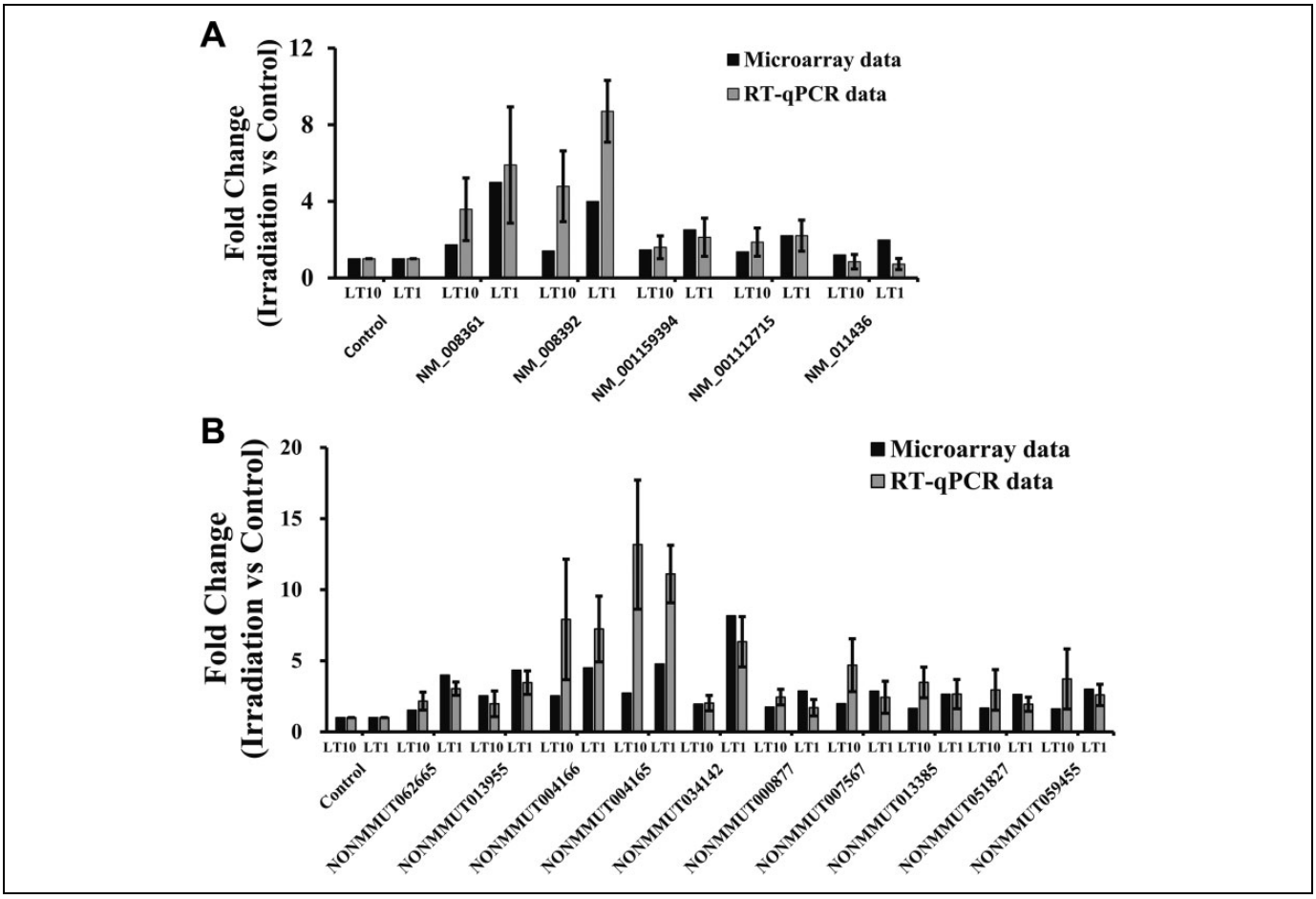

Figure 3. The differential expressions of 5 mRNAs (A) and 10 lncRNAs (B) in the 0.05 Gy×10 and 0.5 Gy×1 irradiated groups were validated by RT-qPCR. The data showed that the expression levels of the lncRNAs and mRNAs were upregulated in the irradiated samples relative to the control mice. The heights of the columns in the chart represent Fold Changes (FCs). Data were presented as mean ± standard deviation. The RT-qPCR results were consistent with the microarray data. LT10 represent 0.05 Gy×10 group and LT1 represents 0.5 Gy×1 group. lncRNA indicates long noncoding RNA; mRNA, messenger RNA; RT-qPCR, real-time quantitative polymerase chain reaction.

84 to 460 lncRNAs and that 1 lncRNA may correlate with 1 to 9 mRNAs in LT10 group, while 1 mRNA may correlate with 99 to 732 lncRNAs and that 1 lncRNA may correlate with 1 to 13 mRNAs in LT1 group (Supplemental Tables S1 and S2). We performed coexpression network to visualize the interaction between the 28 same but differentially expressed lncRNAs correlated with $\geq$7 mRNAs and the 15 same mRNAs in LT10 and LT1 groups (Figure 6). The whole coexpression network consisted of 43 network nodes and 202 connections in LT10, while 43 network nodes and 243 connections in LT1 among the 15 mRNAs and 28 lncRNAs with Pearson correlation coefficients $\geq$0.9 and P value <.05. The number of positive and negative interactions within the network was 139 63 in LT10 group, while 194 49 in LT1 group (Figure 6). There were 15 same positively correlated lncRNAs (eg, uc029vjs.1, NONMMUT035971, NONMMUT067246, NONMMUT019442, NONMMUT004119, NONMMUT016222, NONMMUT040877, NONMMUT005992, ENSMUST00000118307, NONMMUT014269, NONMMUT034947, NONMMUT056736, ENSMUST00000117104, NONMMUT072239, and KnowTID_00005687) and 1 same negatively correlated lncRNA (NONMMUT002099) with the 15 mRNAs enriched in immune signaling pathway within the 2 coexpression networks.

Discussion

Although there are some conflicts about whether chronic low-dose exposure (LDIR) is beneficial to human health in previous studies, it is evident that HDIR results in immune suppression, while LDIR modulates a variety of immune responses that have exhibited the properties of immune hormesis.[1,3,4] During the last decades, the clinical potential of LDIR in the treatment of immune-related diseases has been widely investigated on different animal models, although the underlying molecular mechanism is not fully understood yet.[7,28] Therefore, we focused on LDIR-mediated immune signaling pathways on the mouse model to understand the molecular mechanism of immune hormesis, which is helpful to further study its potential clinical applications.

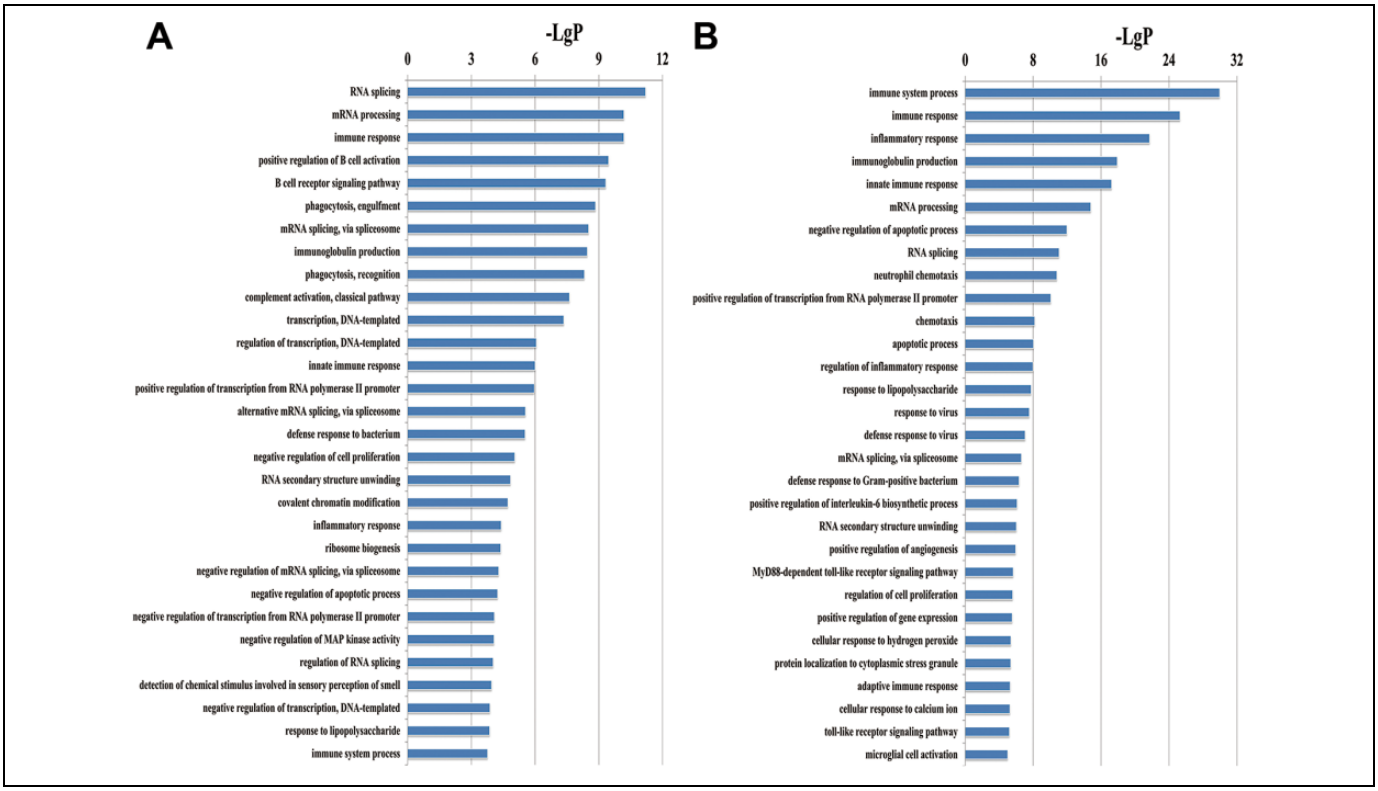

Figure 4. Top 30 biological processes of GO analysis of upregulated mRNAs in the 0.05 Gy×10 (A) and 0.5 Gy×1 (B) irradiated groups compared to the sham-irradiated control group. GO, gene ontology; mRNA, messenger RNA.

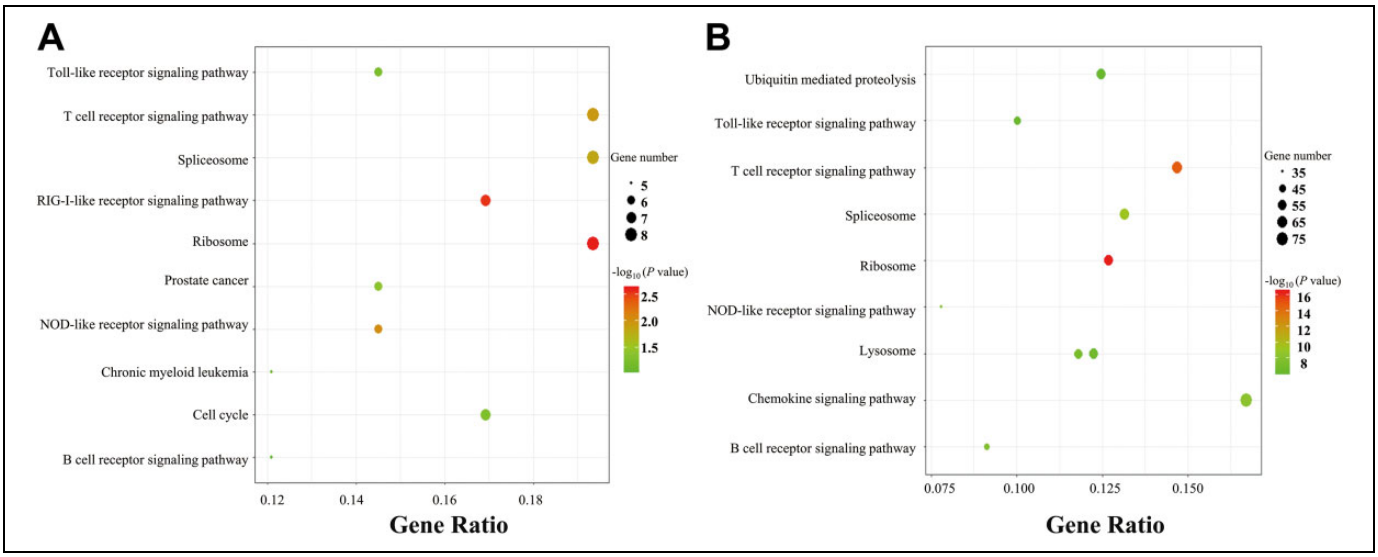

Figure 5. Top 10 signaling pathway of KEGG analysis of upregulated mRNAs in the 0.05 Gy×10 (A) and 0.5 Gy×1 (B) irradiated groups compared to the sham-irradiated control group. KEGG indicates Kyoto Encyclopedia of Genes and Genomes; mRNA, messenger RNA.

Long noncoding RNAs, as extremely critical molecules, are involved in the regulation of various biological processes, such as gene transcription, chromatin modulation, and protein folding and assembly.[29] The accumulating evidence showed the expression of lncRNAs possessing central regulatory function was altered in disease states[30,31] or response to ambient stress, such as salinity stress,[32] oxidative stress,[33] and IR.[19,20] Therefore, analyzing the expression profiles of lncRNAs may provide new insights into our understanding of the molecular mechanism of the biological effects induced by the irradiation using DNA microarray and bioinformatics and discover some lncRNAs as potential valuable candidate biomarkers for

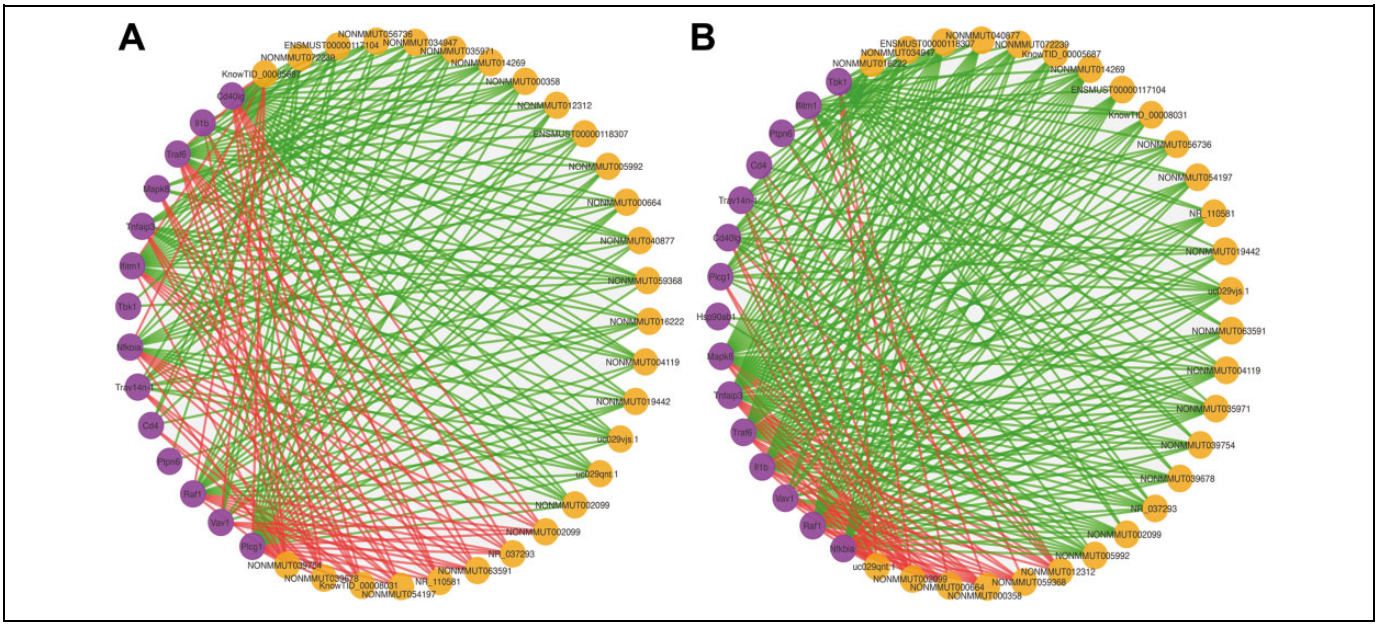

Figure 6. LncRNAs-mRNAs coexpression network analysis among the 15 mRNAs and 28 lncRNAs. Purple nodes represent differently expressed mRNAs, orange nodes represent lncRNAs. The red lines between lncRNAs and mRNAs indicate a negative correlation, while the green lines indicate a positive correlation. lncRNA indicates long noncoding RNA; mRNA, messenger RNA.

radiation biodosimetry. Aryankalayil et al found 2 radiation-induced lncRNAs involved with the immune response as potential biomarkers by observing significant alterations in the expression patterns of lncRNAs across time points and doses in a mouse model after whole-body irradiation.[19] Yang et al elucidated the potential role of lncRNAs in radiation-induced DNA damage basing on the expression profiles of mRNA and lncRNA in 293T cells with or without 8 Gy irradiation using high-throughput sequencing and bioinformatics methods.[34] Beer et al demonstrated lncRNAs played a crucial role in the complex regulatory machinery activated in response to irradiation by analyzing the microarray data of human PBMCs exposed to the irradiation.[35] Tang et al certified that lncRNAs coordinated the tissue response to LDIR exposure via regulation of coding mRNAs using integrated analysis on the microarray data of mammary glands of BALB/c and SPRET/EiJ mice after LDIR exposure.[20] However, to the best of our knowledge, the roles of lncRNAs in the immune response to LDIR have not yet been characterized. This study is the first to describe the correlations between lncRNAs and the immune responses to LDIR using integrated analysis based on the microarray profiles of lncRNAs, which furthered our understanding of the molecular mechanisms in immune responses induced by LDIR.

In this present study, we used DNA microarray and bioinformatics to investigate the profiles of lncRNA and mRNA from PBMC of BALB/c mice at 24 hours after γ-radiation Co^{60} with 0.05 Gy for 10 times (LT10) and 0.5 Gy for 1 time (LT1). Of these, 796 mRNAs and 2077 lncRNAs were detected as differentially expressed for LT10 group, while 7240 mRNAs and 8274 lncRNAs were observed as differentially expressed for LT1 group (fold-change ≥ 1.1, $P < .05$). The expressions of 524 mRNAs and 1234 lncRNAs were changed in the both irradiation groups compared to sham-irradiated group. In addition, the 15 lncRNAs and 5 mRNAs were randomly chosen for qRT-PCR validation, and the results confirmed the microarray analysis findings to some extent. Regarding the previous studies, a total of 357, 480, and 335 lncRNAs were identified to be differentially expressed at weeks 2, 4, and 8 after LDIR (0.1 Gy) in comparison to sham (fold-change ≥ 1.5; $P < .05$) in BALB/c mice; however, they did not study the effect of chronic LDIR on the expression profiles of lncRNAs.[20] Taken the single and chronic LDIR in our study, our findings will likely lead toward a better understanding of the function of lncRNAs in biological effects induced by LDIR.

To investigate the biological effects response to LDIR, GO and KEGG pathway analyses were first performed using the coding genes associated. Interestingly, we found that the most enriched GO terms were significantly associated with immune processes (eg, immune response, inflammatory response, immunoglobulin production) based on the upregulated genes in both single and chronic LDIR (Figure 4). The KEGG pathway analysis also indicated that the differently expressed genes in LT10 and LT1 groups were both mainly involved in immune signaling pathways (eg, Toll-like receptor, NOD-like receptor, T-cell receptor, B-cell receptor signaling pathway) in the top10 signaling pathways (Figure 5). The results indicated the both single and chronic LDIR could activate immune responses and strengthen immunity, which were consistent with previous reports. For example, LDIR could enhance $CD4^+$ T-cell responsiveness by modulating these signaling networks through the alteration of expression levels of several T-cell

surface markers and chemokines.[36] Low-dose ionizing radiation could enhance B lymphoblast proliferation by altering the expression of cell cycle proteins and modulate B-cell differentiation through the activation of nuclear factor-kappa B and the induction of cell differentiation molecule CD23 expression.[37] It has been demonstrated that LDIR strengthens the immune response by altering immune cell populations, releasing surface functional molecules and cytokines, and enhancing the function of immune cells.[38-40]

In the present study, to investigate the relevance between differently expressed lncRNAs and LDIR-induced immune signaling pathways, we also employed an lncRNA-mRNA network analysis to identify interactions between 1234 same differentially expressed lncRNAs and 15 same differentially expressed mRNAs enriched in the same immune signaling pathways between LT10 and LT1 groups, as previously described.[34,41] The whole coexpression network consisted of 1056 network nodes and 3365 connections in LT10 group with Pearson correlation coefficients $\geq$0.9 and P value <.05, while 1104 network nodes and 7050 connections in LT1 group. We visualized the connections between the 28 same differentially expressed lncRNAs correlated with $\geq$7 mRNAs and the 15 same mRNAs in LT10 and LT1 groups through drawing coexpression network (Figure 6). Twelve lncRNAs were differently interacted with 15 mRNA between the LT10 and LT1 groups (Figure 6). The results indicated that the molecular mechanisms of the regulation of immune signaling pathway may be different between the single and chronic LDIR induction. It helps to explain why immune-related factors are differently regulated and different immune cell populations are activated in the chronically low-dose-rate-irradiated mice and single-dose-rate-irradiated mice given the same total doses.[38,42] We discovered 15 same lncRNAs were positively interacted and 1 same lncRNA were negatively interacted with 15 mRNA enriched in immune signaling pathway within the 2 coexpression networks, indicating that these lncRNAs might play a crucial role in the immune signaling pathways triggered by LDIR (Figure 6). Although there are limited data on their function and mechanisms as lncRNA biology is both new and complex, the potential functions of lncRNAs in the irradiation-induced responses of several mouse tissues have been successfully predicted by the lncRNAs-mRNAs coexpression network.[20,21] It is clear that knockout or overexpression of the lncRNAs in mice and immune cells should be performed in order to elucidate the regulatory mechanisms of immune hormesis in response to LDIR, as well as the health implications after occupational, environmental, and clinical low-dose exposures.

Conclusions

In this study, we revealed for the first time the profile of differentially expressed lncRNAs and their co-expressed coding genes involved in the responses of the single and chronic LDIR through the integrated analysis of microarray data. First, we discovered that both single and chronic LDIR can trigger immune responses by GO functional and pathway enrichment analysis. In addition, it is suggested that lncRNAs may act as coregulators to regulate gene transcription in immune signaling pathways response to LDIR or to affect the expression of correlative coding genes by the lncRNAs-mRNAs coexpression network. Although these findings provide newly found information regarding the potential role of lncRNAs in the immune response to the LDIR, considering that the researches of these lncRNAs are desperately lacking up-to-date, we are just at the beginning of this researches. Further studies are required to fully clarify the significance of lncRNAs in response to LDIR, which may further deepen our understanding of the biological and molecular mechanisms in the immune responses of the LDIR.

Authors' Note

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Supplemental Material

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