The Adaptive Responses in Non-Small Cell Lung Cancer A549 Cell Lines Induced by Low-Dose Ionizing Radiation and the Variations of miRNA Expression

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Abstract
Objective: To study the effects of adaptive response in A549 cells induced by low-dose radiation and the miRNAs expression.

Methods: A549 cells were irradiated with 50 mGy and 200 mGy initial doses, respectively, and then irradiated with a challenge dose 20 Gy at 6 hours interval. The biological effects and miRNA expression were detected.

Results: The apoptosis rates of 50 mGy-20 Gy and 200 mGy-20 Gy groups were significantly lower than that of only 20 Gy irradiation group (P < .05). The percentage of G2/M phase cells of 50 mGy-20 Gy and 200 mGy-20 Gy groups was significantly decreased relative to the 20 Gy group (P < .05). One miRNA (mir-3662) was upregulated and 15 miRNAs (mir-185, mir-1908, mir-307, mir-182, mir-92a, mi-r501, mir138-5p, mir-1260, mir-484, mir-378d, mir-193b, mir-127-3p, mir-1303, and mir-654-5p) were downregulated both in 50 mGy-20 Gy and 200 mGy-20 Gy groups than that of the 20 Gy group. Go and KEGG enrichment analysis showed that the target genes were significantly enriched in cell communication regulation, metabolic process, enzyme binding, and catalytic activity signaling pathways.

Conclusion: Low-dose X-ray of 50 mGy and 200 mGy radiation can induce adaptive apoptosis response prior to 20 Gy in A549 cells. Sixteen differently expressed miRNAs may play important roles in the adaptive effect of low-dose radiation.

Keywords
low-dose radiation, X-ray, adaptive effect, apoptosis, miRNAs

Introduction
It is well known that low-dose ionizing radiation (LDR) can induce remarkably different biological effects than that of the effects induced by high-dose ionizing radiation (HDR). But the opinion about the effects of LDR is controversial. It centers on whether there is a threshold to the dose-response curve, as not only there could be a threshold for the induction of cancer, but there could also be a protective effect at LDR.1 The linear no-threshold (LNT) model implies that no dose of radiation is safe and continues to be recommended by both the International Commission on Radiological Protection and the National Research Council of the U.S. National Academy of Sciences.2,3 But up to now, various biological effects induced by LDR have been reported, including bystander effects,
hyper-radiosensitivity (HRS), and adaptive response.\(^4\) Of these effects, adaptive response describes the ability of LDR to induce cellular changes that alter the level of subsequent radiation-induced damages. Some studies have shown that adaptive response can enhance immune response, resulting in anti-tumor effects. Pre-exposure to LDR in the range of about 1 cGy reduced the frequency of micronuclei in binucleated cells induced by 100 cGy, modulating the radio-adaptive responses in human lymphocytes.\(^7\) However, other studies have shown that LDR increased radio-resistance responses (IRR) in cells.\(^8\) Clearly, all of the models for the LDR responses are not fully understood.

Lung cancer is the leading cause of cancer mortality in both men and women in China and the lung is a sensitive organ to radiotherapy damage. Liang et al examined the role of LDR in human embryonic lung fibroblast 2BS and small lung cancer NCI-H446 cell lines. They found that LDR stimulates cell proliferation via the activation of both MAPK/ERK and PI3K/AKT signaling pathways in 2BS but not in NCI-H446 cells.\(^9\) To further explore the effects of LDR on lung cancer cells, in the present study, we detected the effects of LDR on apoptosis and cell cycle in lung cancer A549 cell line and screened the relative miRNAs using small RNA sequencing.

**Materials and Methods**

**Cell Culture**

Lung cancer A549 cells were cultured in DMEM (GIBCO) supplemented with 10% fetal bovine serum (FBS) (GIBCO) at 37°C under 5% CO2 in a humidified incubator.

**Groups and Radiation**

The A549 cells were divided into 6 groups: 0 Gy (C), 50 mGy (LDR1), 200 mGy (LDR2), 50 mGy-20 Gy (LDR1_H), 200 mGy-20G (LDR2_H) y, and 20 Gy (HDR) radiation groups. The cells were trypsinized and cultured in T25 cell culture plates for 12 hours. Then, these cells were irradiated using the equipment of X-RAY 225 (Precision X-ray, USA) with different dose at a rate of .038 Gy/min: 0 Gy for control group, 50 mGy for LDR1 group, and 200 mGy for LDR2 group, respectively. For the LDR1_H and LDR2_H groups, cells were first irradiated with 50 mGy and 200 mGy doses, respectively. Then, these cells were additionally irradiated with the dose of 20 Gy at a rate of .096 Gy/min after 6 hours. All the following experiments were carried out according to these groups.

**Cell Apoptosis Assay**

Cells were harvested 24 hours post irradiation for apoptosis detection using the annexin V-FITC apoptosis detection kit (Sigma, USA) and subsequently analyzed by Accuri C6 Plus flow cytometry (BD, USA).

**Cell Cycle Assay**

Cells were harvested 48 hours post irradiation for cell cycle profiles examination using flow cytometry. Briefly, cell monolayers were washed with phosphate-buffered saline (PBS), trypsinized and resuspended in ice-cold PBS. Cells were then gently pelleted by centrifugation (300×g for 5 min at 4°C), the supernatant was removed and the cells were fixed and permeabilized by the dropwise addition of 70% ethanol at −20°C while vortexing. Fixed cells were washed with PBS and incubated in the dark for 30 min with a propidium iodide (PI) staining solution containing 50 μg/ml propidium iodide (PI) and 100 μg/ml RNase A in PBS. The propidium iodide fluorescence per cell was measured with a flow cytometer (BD, Franklin Lakes, NJ) equipped with a 488 nm argon laser. The width (FL2W) and area (FL2A) of the PI fluorescence per cell were recorded for at least 10 000 cells per sample. Histograms of the PI intensities were plotted. The percentage of cells in each phase of the cell cycle was analyzed using ModFit software.

**RNA Extraction**

Total RNA was extracted from A549 cells using the Absolutely RNA™ RT-PCR Miniprep kit (Stratagene), according to the manufacturer’s instructions. Total RNA concentration was adjusted to 2 ng/μL using a spectrophotometer.

**RNA Quantification and Qualification**

RNA degradation and contamination was monitored on 1% agarose gels. RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA). RNA concentration was measured using Qubit® RNA Assay Kit in Qubit® 2.0 Fluorometer (Life Technologies, CA, USA). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

**Library Preparation for Small RNA Sequencing**

A total amount of 3μg total RNA per sample was used as input material for the small RNA library. Sequencing libraries were generated using NEBNext® Multiplex Small RNA Library Prep Set for Illumina® (NEB, USA) following manufacturer’s recommendations and index codes were added to attribute sequences to each sample. Briefly, NEB 3’ SR Adapter was directly and specifically ligated to 3’ end of miRNA, siRNA, and piRNA. After the 3’ ligation reaction, the SR RT Primer hybridized to the excess of 3’ SR Adapter (that remained free after the 3’ ligation reaction) and transformed the single-stranded DNA adapter into a double-stranded DNA molecule. This step was important to prevent adapter-dimer formation, besides, dsDNAs were not substrates for ligation mediated by T4 RNA Ligase 1 and therefore did not ligate to...
the 5’ SR Adapter in the subsequent ligation step. 5’ ends adapter was ligated to 5’ ends of miRNAs, siRNA, and piRNA. Then, first strand cDNA was synthesized using M-MuLV Reverse Transcriptase (RNase H). PCR amplification was performed using LongAmp Taq 2X Master Mix, SR Primer for illumina and index (X) primer. PCR products were purified on an 8% polyacrylamide gel (100V, 80 min). DNA fragments corresponding to 140~160 bp (the length of small non-coding RNA plus the 3’ and 5’ adapters) were recovered and dissolved in 8 µL elution buffer. At last, library quality was assessed on the Agilent Bioanalyzer 2100 system using DNA High Sensitivity Chips.

**Clustering and Sequencing**

The clustering of the index-coded samples was performed on a cBot Cluster Generation System using HiSeq Rapid Duo cBot Sample Loading Kit (Illumina, NEB, USA) according to the manufacturer’s instructions. After cluster generation, the library preparations were sequenced on an Illumina Hiseq 2500 SE50 platform and 50 bp single-end reads were generated.

**Quality Control**

Raw data (raw reads) of fast q format were first processed through custom perl and python scripts. In this step, clean data (clean reads) were obtained by removing reads containing ploy-N, with 3’/adapter contaminants, without 3’/adapter or the insert tag, containing ploy A or T or G or C, and low quality reads from raw data. At the same time, Q20, Q30, and GC content of the raw data were calculated. Then, we chose a certain range of length from clean reads to do all the downstream analyses.

**Target Prediction and Bioinformatics Analysis of miRNAs**

Prediction of the target-related cellular pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and DAVID (Database for Annotation, Visualization and Interrogation Discovery, http://david.abcc.ncifcrf.gov/home.jsp) bioinformatics resources v6.7 according to the developer’s protocol, and categorization of target genes with specific biological functions using the AmiGO Gene Ontology (GO) analysis tool (http://amigo.geneontology.org/amigo). The prediction of target genes was limited by setting the value of the threshold to .8 in the DIANA-microT tool. The “KEGG_ pathway” category was processed by setting the threshold of EASE score, a modified Fisher Exact P-value, to .1, and involved KEGG pathways that displayed a value >1% (percentage of involved target genes/total target genes in each pathway) were selected. GO analysis was performed in 8 categories for positive or negative regulation of apoptotic processes, cell growth, cell proliferation, and cell cycle.

**Statistical Analysis**

All statistical analysis was performed using SPSS19.0 software. Results were statistically evaluated using Chi-square test. \( P < .05 \) was considered to be statistically significant.

**Results**

**The Effects of LDR as a Pre-adapting Dose on the Apoptosis of A549 Cell**

A549 cells were irradiated with 50 mGy and 200 mGy initial doses, respectively, and then irradiated with 20 Gy at 6 hours interval. The apoptosis of A549 cells was detected by flow cytometry after 24 hours of culture. Compared with 0 Gy group, the apoptosis rates of other 5 groups were significantly higher \( (P < .05) \) (Figure 1). No significant differences were observed between 50 mGy vs 200 mGy and 50 mGy-20 Gy vs 200 mGy-20 Gy groups. But the apoptosis rates of 50 mGy-20 Gy and 200 mGy-20 Gy groups were significantly lower than that of only the 20 Gy irradiation group \( (P < .05) \) (Figure 1), showing that the dose of 50 mGy and 200 mGy both can induce the adaptive response of A549 cells.

**The Effects of LDR as a Pre-adapting Dose on the Cell Cycle of A549 Cell**

Compared with 0 Gy group, the percentage of G2/M phase cells in 50 mGy and 200 mGy groups had no significant difference. But the significant increasing were found in 50 mGy-20 Gy, 200 mGy-20 Gy, and 20 Gy groups comparing with 0 Gy group, respectively \( (P < .05) \). Meanwhile, the percentage of G0/G1 phase cells in 50 mGy-20 Gy, 200 mGy-20 Gy, and 20 Gy groups decreased accordantly \( (P < .05) \).
Importantly, compared with 20 Gy group, the percentage of G2/M phase cells both in 50 mGy-20 Gy and 200 mGy-20 Gy groups were significantly decreased ($P < .05$) (Figure 2), showing that LDR given before a HDR challenge resulted in a lower fraction of cells being damaged and halted by the G2/M checkpoint(s).

The Effects of LDR as a Pre-adapting Dose on the Expression of miRNAs

To further explore the possible mechanisms, we detected the expression difference of miRNA using small RNAs sequencing. 875, 889, 649, 681, and 905 mature miRNAs were observed to be differentially expressed in comparison with the 0 dose control group, respectively (Table 1). The number of up-regulation or down-regulation miRNAs that statistically significant changes >2-fold log value in every comparing groups were: 50 mGy vs 0 Gy group, 14 up-regulation, 30 down-regulation (Figure 3a). 200 mGy vs 0 Gy group, 26 up-regulation, 62 down-regulation (Figure 3b). 20 Gy vs 0, 41 up-regulation, 24 down-regulation (Figure 3c). 50 mGy-20 Gy vs 20 Gy group, 7 up-regulation and 36 down-regulation (Figure 3d). 200 mGy-20 Gy vs 20 Gy group, 23 up-regulation, and 40 down-regulation (Figure 3e). Compared with 20 Gy group, the co-up-regulation miRNAs was 1 and co-downregulation miRNAs were 15 in 50 mGy-20 Gy and 200 mGy-20 Gy groups (Figure 3f,g).

The sequencing results of small RNAs showed that these 16 miRNAs were miR-3662, miR-185, miR-1908, miR-1307, miR-182, miR-92a, miR-582, miR-501, miR-138-5p, miR-1260, miR-484, miR-378d, miR-193b, miR-127-3p, miR-1303, and miR-654-5p, respectively. Among these miRNAs, miR-3662 was up-regulated and others were down-regulated both in 50 mGy-20 Gy and 200 mGy-20 Gy irradiation.
groups compared with the 20 Gy group (Table 2). Then, the qRT-PCR was used to validate the expression of these miRNAs in 0 Gy and 50 mGy-20 Gy groups, which was consistent with that of microarray sequencing results (Figure 4).

Table 1. Different expression of miRNAs comparing with control group.

| Group    | Dose   | Total miRNAs | Mature miRNAs |
|----------|--------|---------------|---------------|
| LDR1     | 50 mGy | 6 726 884     | 875           |
| LDR2     | 200 mGy| 6 224 200     | 889           |
| LDR1_H   | 50 mGy-20 Gy | 6 636 736 | 649           |
| LDR2_H   | 200 mGy-20 Gy | 5 800 299 | 681           |
| HDR      | 20 Gy  | 17 837 474    | 905           |

Figure 2. The effects of LDR as a pre-adapting dose on the cell cycle of A549 cell. (A) The results of A549 cells cycle induced by different dose radiation. (B) The histogram of the cell cycle experiments. Results correspond to the mean ± SD of experiments, performed in triplicate in each case. (A) compared with 0 Gy group, \( P < .05 \). (B) compared with 20 Gy group, \( P < .05 \).

Table 1. Different expression of miRNAs comparing with control group.

The Go Ontology and KEGG Signal Pathway Analysis for Target Genes of Different Expression miRNAs

Go ontology enrichment analysis showed that the target genes regulated by these differently expressed miRNAs were significantly enriched in biological processes, cellular component, and molecular functions. Different biological processes mainly included regulation of cell communication, positive of regulation of metabolic process, regulation of signaling metabolic process. Cellular component included cytoplasm and cytoplasmic part, intracellular and intracellular part. Molecular functions included protein binding enzyme binding and catalytic activity (Figure 5). KEGG pathway analysis showed that these targets genes mainly centralized in endocytosis, VEGF signaling pathway, Ras Signaling pathway, RAP1 signaling pathway, cell communication regulation,
metabolic process, enzyme binding and catalytic activity, lysosome, and MAPK signaling pathway (Figure 6).

**Discussion**

An LDR adaptive response is characterized by the induction of radio resistance to subsequent high doses of radiation through LDR pretreatment. Up to now, many studies have reported that LDR can induce adaptive response in vivo and vitro. Elmore et al concluded that doses of less than 100 mGy delivered at very low dose rates in the range 1 to 4 mGy/day can induce an adaptive response against neoplastic transformation in vitro. When the dose rate drops below 1 mGy/day, this suppression is apparently lost, suggesting a possible dose-rate–dependent threshold for this process. Both cell-proliferation hormesis and cell-survival adaptive response to subsequent high-dose radiation in bone marrow cells induced by LDR were reported. To further explore if LDR can induce adaptive response, in the present study, we detect the effects of LDR on the apoptosis, and cell cycle of A549 cells. Given that The United Nations Scientific Committee on the Effects of Atomic Radiation the Committee (UNSCEAR) defined low doses, as those of 200 mGy or less and low dose rates as .1 mGy/min or less for low-LET radiation in the past, but now has agreed low
doses be defined as those of 100 mGy or less. This definition is consistent with that used by ICRP and the BEIR VII report, so the dose of 50 mGy and 200 mGy were used in the present study. The results showed that the apoptosis rates of 50 mGy-20 Gy and 200 mGy-20 Gy groups were significantly lower than that of only 20 Gy irradiation group, showing that the dose of 50 mGy and 200 mGy both can induce the adaptive response of A549 cells. But in other studies, adaptive response was not observed in cancer cells. Jiang et al found that LDR induced adaptive response in normal lung epithelial cells but not in lung cancer cells. Four tumor cell lines: 2 human leukemia cell lines and 2 human solid tumor cell lines, along with 1 normal cell line were irradiated with LDR at 75 mGy of X-rays as D1 and then 4 Gy of X-rays as D2 (i.e., D1 + D2) or only 4 Gy of X-rays (D2 alone). Three tumor-bearing animal models were also used to further define whether LDR induces adaptive response in tumor cells in vivo. Adaptive response was observed only in normal cell line, but not in 4 tumor cell lines, in response to LDR, showing a resistance to subsequent D2-induced cell growth inhibition. We speculated that the reason which led to different results may owe to the different effective high dose used in these experiments and LDR affects cell in a cell-type-dependent manner. The different adaptive responses of individuals suggested that different cell types also exhibited different adaptive responses. The specific mechanism should be further explored in future studies. LDR was previously shown to enhance cell protection through activation of the AKT (protein kinase B, PKB)/nuclear factor-kappa B (NFκB) pathway. Apoptotic gene expressions such as Bcl-2 and BAX also can be modified by LDR. Studies showed that the differential sensitivity of non-tumor and tumor cells to low-dose and high-dose radiation depends on

| miRNA ID  | Location | 20 Gy(A) | 50 mGy-20 Gy (B) | 200 mGy-20 Gy(C) | Log2(B/A) | Log2(C/A) |
|-----------|----------|----------|------------------|------------------|-----------|-----------|
| hsa-miR-92a | 13q31.3  | 7712.85  | 3157.42          | 3396.89          | -1.29     | -1.19     |
| hsa-miR-193b | 16p13.12 | 1291.15  | 523.84           | 379.80           | -1.30     | -1.77     |
| hsa-miR-484 | 16p13.11 | 572.06   | 210.49           | 256.39           | -1.44     | -1.16     |
| hsa-miR-1307 | 10q24.33 | 9.66     | 0                | 1.06             | -3.60     | -3.19     |
| hsa-miR-182 | 7q32.2   | 7.11     | 0                | 0                | -3.83     | -3.83     |
| hsa-miR-127-3p | 14q32.2 | 652.59   | 326.11           | 297.8            | -1.00     | -1.14     |
| hsa-miR-1303 | 5p33.2   | 106.58   | 47.04            | 25.53            | -1.18     | -2.07     |
| hsa-miR-138-5p | 3p21.22/16p13.3 | 54.29   | 15.15            | 10.64            | -1.84     | -2.36     |
| hsa-miR-185 | 22q11.21 | 42.63    | 8.77             | 7.45             | -2.28     | -2.52     |
| hsa-miR-654-5p | 14q32.31 | 32.25    | 15.95            | 10.64            | -1.02     | -1.61     |
| hsa-miR-3662 | 6q23.3   | 2.00     | 26.31            | 15.96            | 3.71      | 2.99      |
| hsa-miR-378d | 4p16.2/8q22.1 | 28.79   | 7.97             | 11.70            | -1.85     | -1.30     |
| hsa-miR-1908 | 11q12.2  | 10.57    | 2.39             | .00              | -2.14     | -4.41     |
| hsa-miR-582 | 5q11.1   | 24.23    | 9.57             | 8.51             | -1.34     | -1.52     |
| hsa-miR-501 | Xp11.23  | 98.38    | 39.07            | 23.40            | -1.33     | -2.08     |
| hsa-miR-1260 | 11q21    | 83.99    | 28.70            | 26.60            | -1.55     | -1.66     |

Figure 4. Validation of sequencing results by qRT-PCR. Triplicate assays were performed for each RNA sample. Data were normalized using U6 as an endogenous control for RNA input. Fold changes for these miRNAs from real-time RT-PCR were shown as mean ± SD.
Figure 5. The Go ontology enrichment analysis results of 4 groups. (A) 50 mGy vs 0 Gy, (B) 200 mGy vs 0 Gy, (C) 20 mGy-20 Gy vs 20 Gy, and (D) 200 mGy-20 Gy vs 20 Gy.

Figure 6. KEGG signal pathway analysis of 4 groups. (A) 50 mGy vs 0 Gy, (B) 200 mGy vs 0 Gy, (C) 20 mGy-20 Gy vs 20 Gy, and (D) 200 mGy-20 Gy vs 20 Gy.
AKT activity. In radiosensitive non-tumor cells lacking AKT activity, chronic low-dose radiation might inhibit cell death induced by cytotoxic high-dose radiation through modulation of the activation level of the ERK pathway. We also detected induced by cytotoxic high-dose radiation through modulation of AKT activity. In radiosensitive non-tumor cells lacking AKT activity. Chronic low-dose radiation might inhibit cell death through modulation of AKT activity. Given that the cells in G2/M phase were more sensitive to radiation than that of other phases, this result showed that LDR can reduce the radiation sensitivity of A549 cells following high-dose radiation through blocking the cell cycle progression.

MicroRNAs (miRNAs) are small non-coding RNA molecules capable of regulating expression of over 50% of protein-coding genes. The effect of miRNAs on adaptive responses induced by LDR has also been reported. Given that the dose of 50 mGy and 200 mGy both can induce the adaptive response of A549 cells, we detected the expression difference of miRNAs. The sequencing results of small RNAs showed that compared with 20 Gy irradiation group, 1 miRNA (miR-3662) was upregulated and 15 miRNAs (miRNA-185, miRNA-1908, miRNA-1307, miRNA-182, miRNA-92a, miRNA-582, miRNA-501, miRNA-138-5p, miRNA-1260, miRNA-484, miRNA-378d, miRNA-193b, miRNA-127-3p, miRNA-1303, and miRNA-654-5p) were downregulated both in 50 mGy-20 Gy and 200 mGy-20 Gy irradiation groups, indicating that these miRNAs may play important role in adaptive response of A549 cells. Then the qRT-PCR was used to validate the expression of miRNAs, the results were consistent with that of microarray sequencing results. Of these miRNAs, miRNA-185 has been reported that they were downregulated after radiation treatment with 2 Gy x-ray. Particularly, miRNA-185 negatively regulates ATR expression at post-transcriptional level, enhances radiation-induced apoptosis, and inhibits proliferation by repressing ATR pathway. Elevation of miR-185 sensitizes renal cell carcinoma cells to X-rays. These results and ours indicated that miRNA-185 not only has effects on HDR but also has an adaptive response induced by LDR. The function of other miRNAs also has been reported in some cancers, but not in adaptive response fields. The molecular basis of gene regulation in cells exposed to ionizing radiation is not fully understood. As each miRNA can regulate hundreds of target genes, it is assumed that the majority of the 20 000-25 000 human genes may be regulated by specific miRNAs (van Kouwenhove et al, 2011). To further explore the mechanism of adaptive response regulated by these miRNAs, the target genes were predicted by GO and KEGG analysis. The results showed that the target genes of these 16 miRNAs were focus on the biological process, cellular components and molecular functions, including cell communication regulation, metabolic process, enzyme binding and catalytic activity, lysosome, MAPK signaling pathway, RAS signaling pathway, endocytosis, and other signaling pathways.

In conclusion, the present study showed that both 50 mGy and 200 mGy LDR X-ray can induce the adaptive response prior to 20 Gy in A549 cells. Go and KEGG enrichment analysis showed that the target genes regulated by these differently expressed miRNAs were significantly enriched in cell communication regulation, metabolic process, enzyme binding and catalytic activity, lysosome, MAPK signaling pathway, RAS signaling pathway, endocytosis, and other signaling pathways. The potential mechanism of these 16 miRNAs regulation adaptive response should be further explored.

Declaration of Conflicting Interests
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References
1. Nowosielska EM, Cheda A, Wrembel-Wargocka J, Janiak MK. Effect of low doses of low-let radiation on the innate anti-tumor reactions in radioresistant and radiosensitive mice. Dose-Response. 2012;10(4):500-515.
2. Veleghzaninov IO, Shadrin DM, Pylina YI, et al. Differential molecular stress responses to low compared to high doses of ionizing radiation in normal human fibroblasts. Dose-Response 2015;13(1):14-058.
3. Zhao X, Cui J-W, Hu J-H, Gao S-J, Liu X-L. Effects of low-dose radiation on adaptive response in colon cancer stem cells. Clin Transl Oncol. 2017;19(7):907-914.
4. Shemetun O, Pilinska MA, Pilinska M. Radiation-induced bystander effect - modeling, manifestation, mechanisms, persistence, cancer risks (literature review). Probl Radiac Med Radiobiol. 2019;24:65-92.
5. Valentini V, Massacci M, Balducci M, et al. Low-dose hyper-radiosensitivity: is there a place for future investigation in clinical settings? Int J Radiat Oncol Biol Phys. 2010;76(2):535-539.
6. Elmore E, Lao X-Y, Kapadia R, Giedzinski E, Limoli C, Redpath JL. Low doses of very low-dose-rate low-LET radiation suppress radiation-induced neoplastic transformation in vitro and induce an adaptive response. Radiat Res. 2008;169(3):311-318.
7. Venkat S, Apte SK, Chaubey RC, Chauhan PS. Radiative response in human lymphocytes in vitro. J Environ Pathol Toxicol Oncol. 2001;20(3):165-175.
8. Marples B, Collis SJ. Low-dose hyper-radiosensitivity: past, present, and future. Int J Radiat Oncol Biol Phys. 2008;70(5):1310-1318.
9. Liang X, Gu J, Yu D, et al. Low-dose radiation induces cell proliferation in human embryonic lung fibroblasts but not in lung cancer cells: importance of ERK1/2 and AKT signaling pathways. *Dose-Response*. 2016;14(1):1559325815622174.

10. Eisen TJ, Eichhorn SW, Subtelny AO, Bartel DP. MicroRNAs cause accelerated decay of short-tailed target mRNAs. *Mol Cell*. 2020;77(4):775-785.

11. Wang G-J, Cai L. Induction of cell-proliferation hormesis and cell-survival adaptive response in mouse hematopoietic cells by whole-body low-dose radiation. *Toxicol Sci*. 2000;53(2):369-376.

12. Jiang H, Li W, Li X, Cai L, Wang G. Low-dose radiation induces adaptive response in normal cells, but not in tumor cells: in vitro and in vivo studies. *J Radiat Res*. 2008;49(3):219-230.

13. Park HS, Seong KM, Kim JY, et al. Chronic low-dose radiation inhibits the cells death by cytotoxic high-dose radiation increasing the level of AKT and acinus proteins via NF-κB activation. *Int J Radiat Biol*. 2013;89(5):371-377.

14. Azimian H, Bahrine-Toossi MT, Rezaei AR, Rafatpanah H, Hamzehloei T, Fardid R. Up-regulation of Bcl-2 expression in cultured human lymphocytes after exposure to low doses of gamma radiation. *J Med Phys*. 2015;40(1):38-44.

15. Park HS, You GE, Yang KH, et al. Role of AKT and ERK pathways in controlling sensitivity to ionizing radiation and adaptive response induced by low-dose radiation in human immune cells. *Eur J Cell Biol*. 2015;94(12):653-660.

16. Bae S, Kim K, Cha HJ, et al. Low-dose γ-irradiation induces dual radio-adaptive responses depending on the post-irradiation time by altering microRNA expression profiles in normal human dermal fibroblasts. *Int J Mol Med*. 2015;35(1):227-237.

17. He J, Tian N, Yang Y, et al. miR-185 enhances the inhibition of proliferation and migration induced by ionizing radiation in melanoma. *Oncol Lett*. 2017;13(4):2442-2448.

18. Wang J, He J, Su F, et al. Repression of ATR pathway by miR-185 enhances radiation-induced apoptosis and proliferation inhibition. *Cell Death Dis*. 2013;4:e699.