J. Radiat. Res., 52, 380–386 (2011)



# The Low-dose Ionizing Radiation Stimulates Cell Proliferation via Activation of the MAPK/ERK Pathway in Rat Cultured Mesenchymal Stem Cells

Xinyue LIANG[1], You Ho SO[1,2], Jiuwei CUI[1], Kewei MA[1], Xiaoyi XU[1], Yuguang ZHAO[1,3], Lu CAI[1,3†] and Wei LI[1*]

**Low-dose ionizing radiation/Hormesis/Cell proliferation/MAPK/ERK pathway/Mesenchymal stem cell.**

Hormesis induced by low-dose ionizing radiation (LDIR) is often mirrored by its stimulation of cell proliferation. The mitogen-activated protein kinases (MAPK)/ extracellular-signal- regulated kinases (ERK) pathway is known to play important roles in cell growth. Therefore, this study was to examine the effects of LDIR on rat mesenchymal stem cell (MSC) proliferation and MAPK/ERK signaling pathway. Rat MSCs were isolated from the bone marrow from 6 to 8-week-old male Wistar rats and cultured *in vitro*. Exponentially growing cells within 4–5 passages were irradiated with low doses of X-rays at 20, 50, 75 and 100 mGy with a dose rate of 100 mGy/min. Cell proliferation was evaluated by counting total viable cell number with trypan-blue staining and MTT assay. Cell cycle changes were also evaluated by flow cytometry and the activation of MAPK/ERK signaling pathway was assayed by Western blotting. Results showed that LDIR at 50 and 75 mGy significantly stimulated the proliferation of rat MSCs with the most stimulating effect at 75 mGy. There was a significant increase in the proportion of S phase cells in MSCs in response to 75 mGy X-rays. Activation of several members in the MAPK/ERK signaling pathway, including c-Raf, MEK and ERK were observed in the cells exposed to 75 mGy X-rays. To define the role of ERK activation in LDIR-stimulated cell proliferation, LDIR-treated MSCs were pre-incubated with MEK specific inhibitor U0126, which completely abolished LDIR-increased phosphorylation of ERK and cell proliferation. These results suggest that LDIR stimulates MSC proliferations in the *in vitro* condition via the activation of MAPK/ERK pathway.

## INTRODUCTION

Low-dose ionizing radiation (LDIR) induced hormesis has been extensively observed in different biological systems including the reproductive, immune, and hematopioetic system.[1–3] Exposure of cells to LDIR stimulates cellular metabolic activities, including DNA, RNA and protein synthesis.[4] By examining mouse hematopoietic progenitor cells, we have shown a significant stimulation of bone marrow progenitor cell proliferation when mice were exposed to LDIR.[5]

As an important component of the hematopoietic system, mesenchymal stem cells (MSCs) have captured a more attention from researchers and physicians.[6,7] The MSCs have been demonstrated as an attracted cell source for tissue-engineering applications, offering significant advantages over other stem cells, due to their easy isolation and expansion from bone marrow aspirates and also their versatility for differentiation into organs and tissues. The MSCs were recently used clinically to treat stroke, myocardial disorders, spinal cord injury and other neurological disorders.[6,7] But the amount of MSCs in the adult bone marrow is small and the ability of proliferation is weak. Therefore, it is crucial to develop new substances to stimulate and expand the MSCs *in vitro* on a large scale.[6,7] There were only a few studies that have examined whether LDIR has stimulating effect on the MSCs, but these studies were mainly performed *in vivo*.[8,9]

*Corresponding author: Phone: +86-431-88782974,
E-mail: drweili@yahoo.com
†Corresponding author: Phone: 502-852-2214,
Fax: 502-852-5634,
E-mail: L0cai001@louisville.edu
[1]Cancer Center at the First Hospital of Jilin University, 71 Xinmin Street, Changchun 130021, China; [2]Institute of Clinical Medicine, Pyongyang Medical University, Pyongyang, DPR Korea; [3]Departments of Pediatrics and Radiation Oncology, the University of Louisville, 570 South Preston Street, Baxter I, Suite 304F, Louisville, KY 40202, USA.
doi:10.1269/jrr.10121

The MAPK/ERK pathway is a signaling cascade from a receptor on the cell surface to the nucleus. The mitogen-activated protein kinase (MAPK) originally called extracellular signal-regulated kinases (ERK). There are several major components of the MAPK/ERK family such as c-Raf, ERK1/2, P38 MAPK and c-Jun N-terminal kinases (JNK). These kinases communicate by adding phosphate groups to others and are involved in the regulation of meiosis, mitosis, postmitotic functions in differentiated cells,[10–12] and various cellular responses under certain conditions.[13] For instance, P38 MAPK and JNK induced cell cycle arrest and apoptosis in response to stress.[14–17] In contrast, ERK1/2 activation is mediated through growth factor tyrosine phosphorylation and activated by Ras to promote cell proliferation.[10–12,18] Suzuki, *et al.* demonstrated that X-ray irradiation at 20 and 50 mGy stimulated the proliferation of normal human diploid cells and human tumor cells and also increased the ERK1/2 phosphorylation. Suppression of ERK1/2 phosphorylation with its inhibitor PD98059 alleviated the enhanced proliferation of normal human cells by LDIR. Furthermore, overexpression of ERK2 in NCI-H1299 human lung carcinoma cells potentiated the stimulating effect of LDIR on cell proliferation.[12] In consistence with this early study, Kim *et al*. also reported that LDIR at 50 mGy stimulated cell proliferation, along with a significant activation of ERK1/2 and p38 MAPK. A specific ERK1/2 inhibitor, U0126, or its siRNA decreased the phosphorylation of ERK1/2 proteins, resulting in a partial suppression of cell proliferation induced by LDIR.[19] These two studies support the involvement of ERK1/2 activation in the cell proliferation induced by LDIR. Despite these studies focusing on the role of MAPK/ERK in normal diploid cell, Chinese hamster fibroblasts and human skin cells in response to LDIR,[10,12] little is known whether LDIR affects this signal pathway, leading to MSC proliferation change, particularly *in vitro*.

In the present study, therefore, we examined the proliferating effects of LDIR on rat MSCs. This study was performed in an *in vitro* condition in order to eliminate systemic indirect effects. Furthermore, we explored whether the MAPK/ERK signal pathway was involved in the cell proliferative effect induced by LDIR in MSCs.

## MATERIALS AND METHODS

### *Cell culture and reagents*

Six to eight-week-old male Wistar rats were used for collecting MSCs. These rats were housed in the Jilin University's Research Resources Center at 22°C with a 12-h light/dark cycle and were provided free access to standard rodent chow and tap water. All animal procedures were approved by the Institutional Animal Case and Use Committee, which is certified by the Chinese Association for Laboratory Animal Care. MSCs were isolated from bone marrow of these rats and then were maintained in low-glucose (1 g/L) Dulbecco's modified Eagle medium (LG-DMEM, Invitrogen, NY, USA) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 μg/mL). We selected LG-DMEM since Pittenger recommended[20] that low levels of glucose can significantly reduce the MSC premature senescence and spontaneous apoptosis and also can increase the rate of MSC proliferation.[21,22] Cultures were maintained at 37°C in a humidified 5% $CO_2$ incubator. The medium was changed every 3 days. The specific MEK1/2 inhibitor U0126 was obtained from Beyotime (Haimen, China).

### *Irradiation*

Exponentially growing cells within 4–5 passages were irradiated with 20, 50, 75 and 100 mGy X-rays at room temperature. X-rays were given for these cells with a Varian Clinac *21EX* X-ray machine operated at 6 MV energy in the presence of SP34 QA phantom polystyrene (C8H8) with an admixture of 2.1% ± 0.2%TiO2 (IBA) filter plates. The dose rate of X-rays was 100 mGy/min. Control groups were treated similarly except for irradiation. After LDIR cells were cultured at 37°C incubator for further experiments.

### *Proliferation and live cell counting*

Cells ($5 \times 10^4$) were seeded into 60 mm petri dish and incubated for 20 h before irradiation. Cells were irradiated with different doses of X-rays and 24 h later cells were digested with trypsin and counted. At 24 h post-LDIR, cells were trypsinized and seeded in 96-well plates for MTT assay to detect cell proliferation. 20 μL MTT solutions were added into each well and incubated at 37°C with 5% $CO_2$ for 3–4 h. Then each well was added with 100 μL dissolving solution (10% SDS, 5% isobutanol and 0.012 M HCl) and continually incubated for additional 12–16 h. The additional incubation of these wells with dissolving solution is used because this step will eliminate the step to remove medium before adding dissolving solution, which avoids the potential loss of cells during medial removal and also significantly reduces the variation of absorption reading due to the variation of cell loss in each well. Finally, absorbance was measured in a microplate reader (Bio-Rad) at 450 nm. The experiments were performed in triplicate and repeated at least three times.

### *Cell cycle analysis*

Cell cycle was analyzed by flow cytometry. For this end, cells were cultured with 10% FBS/LG-DMEM, and then the medium was changed to 2% FBS/LG-DMEM for another 24 h before irradiation. Cells were harvested at 2, 6 and 24 h after LDIR, and fixed with ice-cold 70% ethanol at 4°C overnight. Then the fixed cells were washed twice with PBS, and stained with 50 μg /mL propidium iodide in the presence of RNase A (10 μg /mL). The stained cells were analyzed for DNA content by fluorescence-activated cell sorting (FACS) in a BD FASCalibur.

*Western blot analysis*

Cells were collected from the 100 mm culture dishes and washed with ice-cold PBS and lysed at 4°C on ice for 20 min in 100 µL lysis buffer (50 mM, pH 7.5 Tris-HCl, 0.25% deoxycholate, 1% Triton X-100 and 0.5 M NaCl). Protein concentrations were determined using Bradford method. Equal aliquots of protein sample (30 µg/lane) were separated by 10% SDS-PAGE gel and transferred onto PVDF membranes. The blot was then incubated 1 h with blocking buffer containing 0.1% Tween 20 and 5% nonfat dry milk. The membrane were incubated with primary antibodies (p-c-Raf, c-Raf, p-ERK1/2, ERK1/2, p-MEK1/2 and MEK1/2, respectively, all which were purchased from Cell Signaling Technology (Beverly, MA, USA) with a dilution of 1:1000 and then with goat anti-rabbit IgG-HRP or goat anti-mouse IgG-HRP (Santa Cruz, CA, USA). The ECL detection system was used to visualize the proteins. Band intensity of repeated western blots was determined by densitometry with ImageJ and the mean value was obtained. For the Western blotting assay, we have performed only one study with combined three separate samples as one to avoid variation among samples within group.

*Statistical analysis*

Data are expressed as means ± standard deviation (SD). Statistical significance between the control and irradiated groups was determined by the student *t* test. Differences with a $P$ value < 0.01 were considered as significant.

## RESULTS AND DISCUSSION

*Effects of LDIR on cell growth and cell cycle*

Effects of LDIR on rat MSCs were investigated by counting viable cells (Fig. 1A) and MTT assay (Fig. 1B). Results showed that cell proliferation was significantly enhanced by LDIR at doses of 50 and 75 mGy compared to the control group and the 100 mGy group. To determine how LDIR affects cell cycle progression, cells were collected at 2, 6,

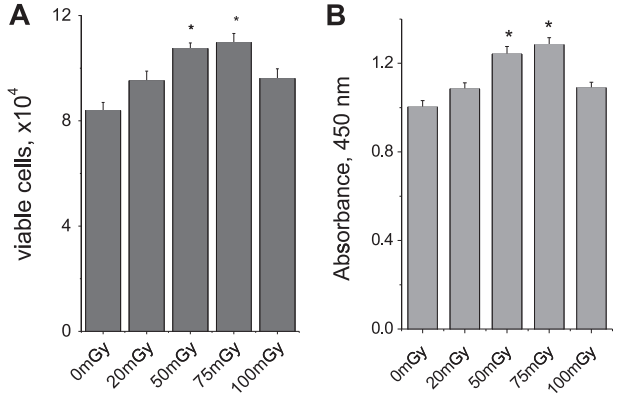


**Fig. 1.** LDIR stimulates rat MSC proliferation. **A**. Cells ($5 \times 10^4$) were seeded in 60 mm petri dishes. The cells received the indicated doses of X-rays and were incubated for an additional 24 h before counting the number of viable cells. **B**. Cell proliferation was determined by MTT assay. *, $P < 0.01$ compared to control (0 Gy group).

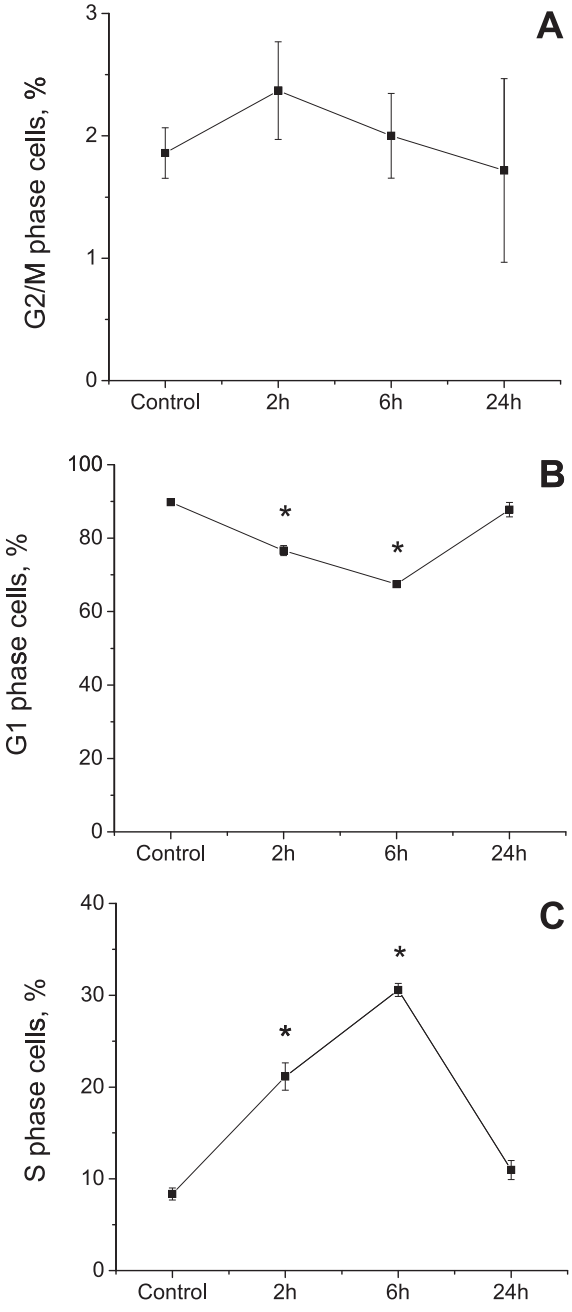


**Fig. 2.** LDIR enhances the number of cells in S phase and reduces the number of cells in G1 phase. The distribution of cells at G2/M (A), G1 (B) and S (C) phases was analyzed at 2, 6, 24 h after irradiation by flow cytometry using propidium iodide staining for DNA content. The graphs represent the percentage of cells in S phase, G1 phase and G2/M phase of the cell cycle, respectively, based on the data from three individual experiments with three replicates per experiment. *, $P < 0.01$ vs control.

24 h following exposure to 75 mGy X-rays and subjected to flow cytometry analysis (Fig. 2A). Data revealed that in the cells exposed to LDIR there was a significant transition from G1 phase to S phase. Quantitative analysis shows that S phase of the cell cycle was significantly increased from 2 to 6 h with an almost 4–7 fold increase in the MSCs after exposure to 75 mGy X-rays (Fig. 2B).

Several previous studies have reported LDIR-increased cell proliferation in normal human diploid cells, Chinese hamster fibroblasts, and Raji lymphoma.[10,12,23] MSCs are known to be capable of regenerating various tissues and are essential in supporting the growth and differentiation of hematopoietic stem cells within the bone marrow microenvironment *in vivo*. To achieve clinically meaningful numbers of cells, therefore, many approaches have been used to maintain the differentiation potentialities and expand enough cells for clinical treatments.[6,7,24] We have shown the stimulating effect on hematopoietic progenitor cells in a mouse model exposed to 75 mGy X-rays,[5] suggesting the potential for the stimulation of MSCs although this was not directly tested. A study reported that low level light irradiation using 630 nm red light emitting diodes could enhance the proliferation of rat MSCs.[9] In addition, Jin *et al.*[25] have investigated the gene transcript profile using human MSC line irradiated with 10, 50, 200 and 1000 mGy γ-rays, examined at 1, 4, 12 and 48 h after exposure. A total of 6,016 genes showed altered expression patterns at more than one time point or dose level among the investigated 10,800 genes. Genes that showed dose-dependent expression responses were involved in signal transduction, regulation of transcription, proteolysis, peptidolysis and metabolism. Among those changed genes, one group was associated with "cellular defense mechanisms" such as apoptosis, cell adhesion, stress response and immune response, while another

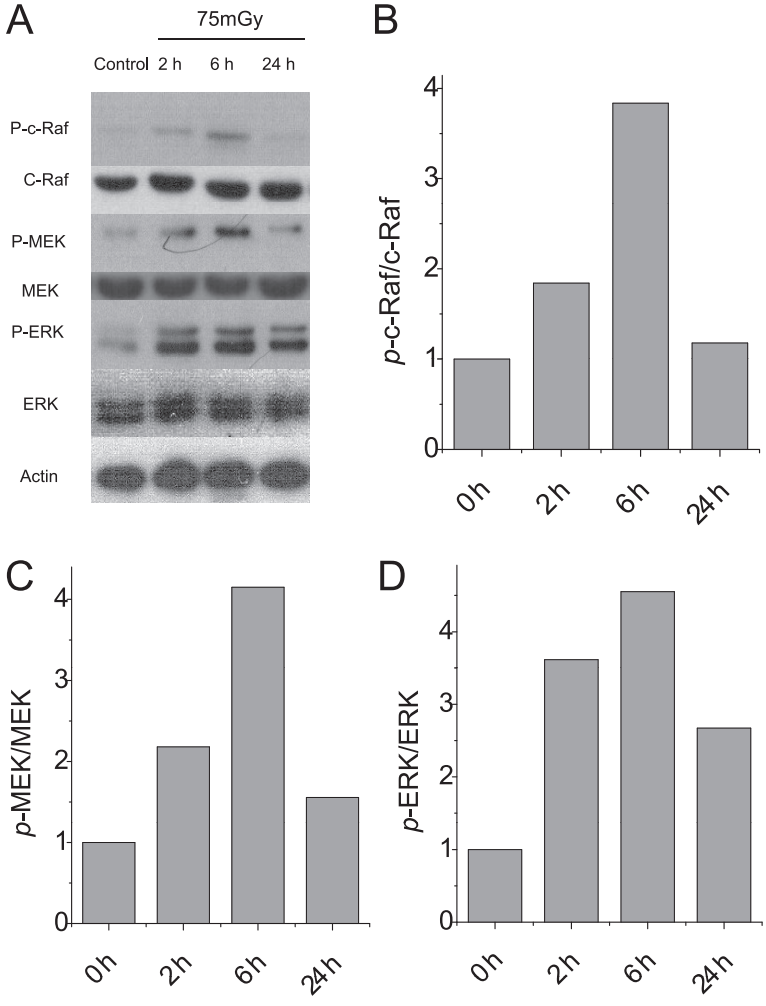


**Fig. 3.** LDIR stimulates phosphorylation of c-Raf, MEK1/2 and ERK1/2 in rat MSCs. **A** Phosphorylation of c-Raf, MEK1/2, and ERK1/2 was examined at 2, 6, 24 h after rat MSCs were irradiated with 75 mGy X-rays by western blot analysis. **B, C, D:** quantitative analysis of the phosphorylated c-Raf, MEK, and ERK1/2 densitometrically based on the blots indicated in panel A.

group was associated with "fundamental cellular processes" such as DNA replication, mitosis, RNA splicing, DNA repair and translation initiation. However, they did not compare these gene changes with biological measurements such as cell proliferation or death. In the present study, therefore, we provide evidence for the first time that 75 mGy X-rays significantly stimulated MSC proliferation, shown by increased total cell numbers and S-phase cells in the cycling cells.

### *Phosphorylation of c-Raf, MEK1/2 and ERK1/2 in the rat MSCs exposed to LDIR*

To determine whether MAPK/ERK signal pathway is involved in LDIR-stimulated cell proliferation, phosphorylation of c-Raf, ERK1/2 and MEK1/2 in the control and 75 mGy X-ray irradiated rat MSCs was examined at the indicated times after irradiation. Representative results from each group are presented in Fig. 3A. Density analysis showed that ratios of phosphorylated c-Raf to total Raf and phosphorylated MEK1/2 to total MEK1/2 were significantly increased from 2 h to 6 h, with a peak increase about 4 fold at 6 h after 75 mGy X-rays (Fig. 3B, C). Similarly the ratio of phosphorylated ERK1/2 to total ERK1/2 was also significantly increased at all observation times, with a peak increase about 4 fold at 6 h after these cells were exposed to 75 mGy X-rays (Fig. 3D).

Several studies showed the important role of Raf-MEK-ERK in improving cell cycling and proliferation.[26] Ahmed *et al.*[27] have provided evidence of the cooperative functions of Ataxia telangiectasia mutated (ATM), ERK, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in inducing cell survival through a radio-adaptive response as a result of LDIR treatment (100 mGy X-rays). By using p53-inhibited human skin keratinocytes, they found that phosphorylation of ATM, MEK, and ERK is enhanced, indicating the p53-independent pro-survival network involving the co-activation of the ATM, MEK/ERK in these cells. Another study using normal human diploid cells also demonstrated that activation of ERK1/2 by 20 mGy X-rays resulted in a stimulation of cell proliferation, suggesting that the activation of ERK1/2 has cell survival effects.[12]

To define whether LDIR-activated ERK1/2 and MEK1/2

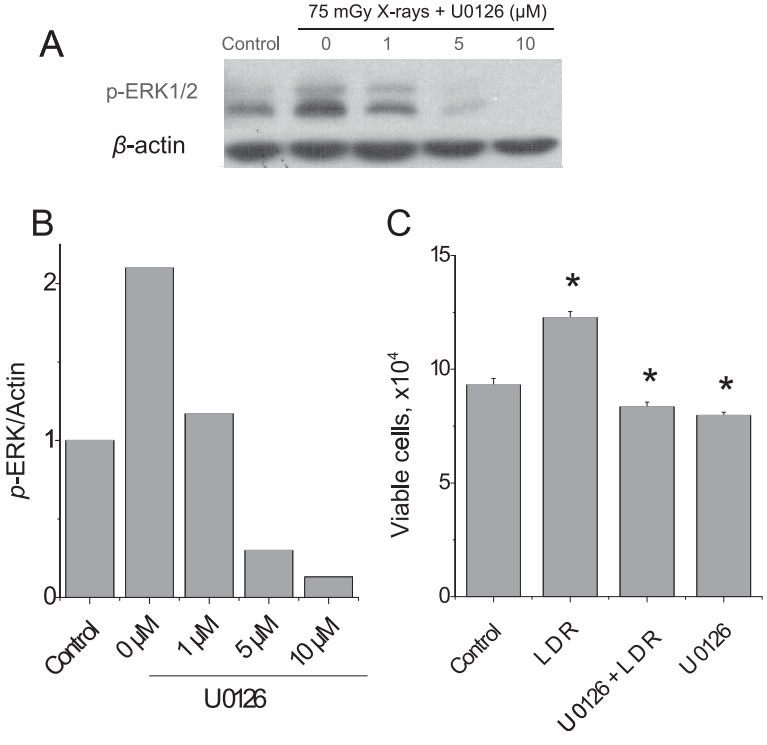


**Fig. 4.** Suppressive effects of MEK inhibitor U0126 on 75 mGy X-rays stimulation of MSC proliferation. **A**. Cells were pretreated with or without 1, 5, 10 μM of MEK inhibitor U0126 for 1 h and then were irradiated with 75 mGy X-rays. From these cells, phosphorylation of ERK1/2 was determined by Western blot analysis at 6 h after LDIR. **B.** Blot was scanned densitometrically, and relative amount of phosphorylated ERK1/2 was calculated against actin expression. **C**. Exponentially-growing cells were pretreated with 10 μM of U0126 for 1 h, and then were irradiated with 75 mGy X-rays. After LDIR, these cells were incubated for additional 24 h before counting. *, $P < 0.01$ vs control.

mediates the LDIR-stimulated MSC proliferation in the present study, we pre-incubated rat MSCs with U0126, a specific inhibitor of MEK, at 1 to 10 μM for 1 h and then irradiated these cells with 75 mGy X-rays, which showed a dose-dependent suppressive effect on ERK1/2 activation (Fig. 4A, B). Pre-incubation with U0126 (1 μM) significantly suppressed the ERK1/2 phosphorylation induced by 75 mGy X-rays down to approximately control level while pre-incubation with U0126 at 5 or 10 μM significantly wiped off the ERK1/2 phosphorylation even lower than those in the control group. This finding suggests that cells have certain amounts of ERK1/2 phosphorylation under normal condition, as showed in Fig. 4A, and U0126 at 5 or 10 μM can even inhibit the normal amounts of ERK1/2 phosphorylation. Therefore, pre-incubation with U0126 at 5 or 10 μM not only completely suppressed the cell proliferating effect in the cells irradiated by 75 mGy X-rays, but also significantly suppressed the cell proliferation of normal cells (Fig. 4C). These results strongly indicate the important role of ERK1/2 activation in the cell proliferation in normal cells or cells irradiated with 75 mGy X-rays.

The MSCs contribute to the regeneration of mesenchymal tissues and are essential in providing support for the growth and differentiation of hematopoietic stem cells within the bone marrow microenvironment *in vivo*. Several studies have shown MSCs able to differentiate into various cell types, including osteocytes, adipocytes, chondrocytes, myocytes, cardiomyocytes, endothelial cells, neurons and hepatocytes *in vitro*.[6,7] Patients who undergo pelvic or abdominal radiotherapy may develop side effects that can be life threatening. Therefore, isolated MSCs from human bone marrow were transplanted into immunotolerent mice as a model of radiation-induced intestinal injury, in which a structural recovery with an increase in small intestinal villus height was achieved at 3 and 15 days following abdominal radiation exposure.[28] This study suggests the potential use of MSC infusion to repair damaged gastro intestinal tract in patients subjected to pelvic radiotherapy. To obtain clinically meaningful numbers of cells, approaches such as genetic immortalization and formulation of growth media have been used to maintain the MSCs' life span and expand to enough numbers of the MSCs for clinical treatment. Few studies have used physical stimulation to enhance the proliferation of stem cells. We have shown the stimulation of bone marrow stem cells and their peripheral mobilization from bone marrow with 75 mGy LDIR *in vivo*. The mononuclear cells collected from 75 mGy-irradiated mice were transplanted to recipient mice irradiated by lethal dose of radiation, and we found the hematological reconstruction in these recipient mice, suggesting the normal function of these stimulated stem cells by 75 mGy X-rays.[5] A recent study showed that low-level light irradiation using 630 nm light emitting diodes could enhance the replication and colony formation potentials of MSCs derived from human or rat bone marrow.[9] Compared to other methods to expand the stem cells, mentioned in the reviews,[6,7] LDIR or low levels of laser irradiation would be more convenient, cheaper, and low risk of stem cells contaminated with the residuals of the reagents that are used to stimulate cell proliferation. Kim *et al*. has reported that LDIR at 50 mGy stimulated cell proliferation along with a significant activation of ERK1/2, which did not change micronuclei frequencies.[19] Whether 75 mGy X-rays can cause any genomic instability on these stems cells remains unclear based on the current study, but this needs to be defined in the future studies.

In summary, we have examined LDIR's effect on the cell proliferation and MAPK/ERK signal transduction in rat MSCs to define whether there is hormesis in the MSCs exposed to LDIR *in vitro*. We found that 75 mGy X-rays can induce a significant increase in proliferation of rat MSCs at 6 h after irradiation, and the stimulation of cell proliferation by 75 mGy X-rays was mediated by up-regulated MEK/ERK signaling pathway since inhibition of MEK function significantly abolished LDIR-induced ERK1/2 activation and LDIR–stimulated cell proliferation.

## ACKNOWLEDGMENTS

We thank Mr. Kunzhi Chen and Ms. Wei Han for their assistances with LDIR exposure for the cells.

## REFERENCES

1. Olivieri G, Bodycote J and Wolff S (1984) Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. Science **223**: 594–597.
2. Liu G, *et al* (2006) Effect of low-level radiation on the death of male germ cells. Radiat Res **165**: 379–389.
3. Ina Y, *et al* (2005) Suppression of thymic lymphoma induction by life-long low-dose-rate irradiation accompanied by immune activation in C57BL/6 mice. Radiat Res **163**: 153–158.
4. Chen SL, *et al* (2000) Low-dose whole-body irradiation (LD-WBI) changes protein expression of mouse thymocytes: effect of a LD-WBI-enhanced protein RIP10 on cell proliferation and spontaneous or radiation-induced thymocyte apoptosis. Toxicol Sci **55**: 97–106.
5. Li W, *et al* (2004) Low-dose radiation (LDR) induces hematopoietic hormesis: LDR-induced mobilization of hematopoietic progenitor cells into peripheral blood circulation. Exp Hematol **32**: 1088–1096.
6. Sensebe L and Bourin P (2009) Mesenchymal stem cells for therapeutic purposes. Transplantation **87**: S49–S53.
7. Liu Zj, Zhuge Y and Velazquez OC (2009) Trafficking and differentiation of mesenchymal stem cells. J Cell Biochem **106**: 984–991.
8. Kurpinski K, *et al* (2009) Differential effects of x-rays and high-energy 56Fe ions on human mesenchymal stem cells. Int J Radiat Oncol Biol Phys **73**: 869–877.
9. Li WT and Leu YC (2007) Effects of low level red-light irra-

diation on the proliferation of mesenchymal stem cells derived from rat bone marrow. Conf Proc IEEE Eng Med Biol Soc **2007**: 5830–5833.
10. Kim CS, *et al* (2007) Low-dose of ionizing radiation enhances cell proliferation via transient ERK1/2 and p38 activation in normal human lung fibroblasts. J Radiat Res (Tokyo) **48**: 407–415.
11. Shin JS, *et al* (2010) Low doses of ionizing radiation suppress doxorubicin-induced senescence-like phenotypes by activation of ERK1/2 and suppression of p38 kinase in MCF7 human breast cancer cells. Int J Oncol **36**: 1445–1452.
12. Suzuki K, Kodama S and Watanabe M (2001) Extremely low-dose ionizing radiation causes activation of mitogen-activated protein kinase pathway and enhances proliferation of normal human diploid cells. Cancer Res **61**: 5396–5401.
13. Narang H, *et al* (2009) Differential activation of mitogen-activated protein kinases following high and low LET radiation in murine macrophage cell line. Mol Cell Biochem **324**: 85–91.
14. Sabatini N, *et al* (2005) JNK/p53 mediated cell death response in K562 exposed to etoposide-ionizing radiation combined treatment. J Cell Biochem **95**: 611–619.
15. Chen YR, *et al* (1996) The role of c-Jun N-terminal kinase (JNK) in apoptosis induced by ultraviolet C and gamma radiation. Duration of JNK activation may determine cell death and proliferation. J Biol Chem **271**: 31929–31936.
16. Assefa Z, *et al* (2000) p38 mitogen-activated protein kinase regulates a novel, caspase-independent pathway for the mitochondrial cytochrome c release in ultraviolet B radiation-induced apoptosis. J Biol Chem **275**: 21416–21421.
17. Bulavin DV, *et al* (1999) Phosphorylation of human p53 by p38 kinase coordinates N-terminal phosphorylation and apoptosis in response to UV radiation. Embo J **18**: 6845–6854.
18. Kornasio R, *et al* (2009) Beta-hydroxy-beta-methylbutyrate (HMB) stimulates myogenic cell proliferation, differentiation and survival via the MAPK/ERK and PI3K/Akt pathways. Biochim Biophys Acta **1793**: 755–763.
19. Kim CS, *et al* (2007) Low-dose radiation stimulates the proliferation of normal human lung fibroblasts via a transient activation of Raf and Akt. Mol Cells **24**: 424–430.
20. Pittenger M (2008) Mesenchymal stem cells from adult bone marrow. In: Darwin J, Donald G and Bruce A eds. *Mesenchymal Stem Cells: Methods and Protocols (Methods in Molecular Biology).* pp. 27–44. Hummana Press.
21. Li YM, *et al* (2007) Effects of high glucose on mesenchymal stem cell proliferation and differentiation. Biochem Biophys Res Commun **363**: 209–215.
22. Stolzing A, Coleman N and Scutt A (2006) Glucose-induced replicative senescence in mesenchymal stem cells. Rejuvenation Res **9**: 31–35.
23. Korystov YN, *et al* (1996) The effect of low-dose irradiation on proliferation of mammalian cells in vitro. Radiat Res **146**: 329–332.
24. Indrawattana N, *et al* (2004) Growth factor combination for chondrogenic induction from human mesenchymal stem cell. Biochem Biophys Res Commun **320**: 914–919.
25. Jin YW, *et al* (2008) Comprehensive analysis of time- and dose-dependent patterns of gene expression in a human mesenchymal stem cell line exposed to low-dose ionizing radiation. Oncol Rep **19**: 135–144.
26. Mccubrey JA, *et al* (2007) Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta **1773**: 1263–1284.
27. Ahmed Km, *et al* (2009) Coactivation of ATM/ERK/NF-kappaB in the low-dose radiation-induced radioadaptive response in human skin keratinocytes. Free Radic Biol Med **46**: 1543–1550.
28. Francois S, *et al* (2006) Local irradiation not only induces homing of human mesenchymal stem cells at exposed sites but promotes their widespread engraftment to multiple organs: a study of their quantitative distribution after irradiation damage. Stem Cells **24**: 1020–1029.

*Received on August 31, 2010*
*Revision received on November 10, 2010*
*Accepted on December 10, 2010*
*J-STAGE Advance Publication Date: March 24, 2011*