

9-2009

MODIFICATION IN THE EXPRESSION OF MRE11/RAD50/NBS1 COMPLEX IN LOW DOSE IRRADIATED HUMAN LYMPHOCYTES

Sompal Singh

Dept of Zoology, M. S. (PG) College, Saharanpur, India

Madhu Bala

Institute of Nuclear Medicine & Allied Sciences, Delhi, India

Raj Kumar

Institute of Nuclear Medicine & Allied Sciences, Delhi, India

Anil Kumar

Institute of Nuclear Medicine & Allied Sciences, Delhi, India

SC Dhiman

Department of Zoology, M. S. (PG) College, Saharanpur, India

Follow this and additional works at: https://scholarworks.umass.edu/dose_response

Recommended Citation

Singh, Sompal; Bala, Madhu; Kumar, Raj; Kumar, Anil; and Dhiman, SC (2009) "MODIFICATION IN THE EXPRESSION OF MRE11/RAD50/NBS1 COMPLEX IN LOW DOSE IRRADIATED HUMAN LYMPHOCYTES," *Dose-Response: An International Journal*: Vol. 7 : Iss. 3 , Article 3.

Available at: https://scholarworks.umass.edu/dose_response/vol7/iss3/3

This Article is brought to you for free and open access by ScholarWorks@UMass Amherst. It has been accepted for inclusion in Dose-Response: An International Journal by an authorized editor of ScholarWorks@UMass Amherst. For more information, please contact scholarworks@library.umass.edu.

MODIFICATION IN THE EXPRESSION OF MRE11/RAD50/NBS1 COMPLEX IN LOW DOSE IRRADIATED HUMAN LYMPHOCYTES

Sompal Singh □ Department of Zoology, M. S. (PG) College, Saharanpur-247 001, India

Madhu Bala, Raj Kumar, and Anil Kumar □ Division of Radiation Biosciences, Institute of Nuclear Medicine & Allied Sciences, Brig SK Mazumdar Marg, Delhi-110 054, India

SC Dhiman □ Department of Zoology, M. S. (PG) College, Saharanpur-247 001, India

□ Despite the fact that high doses of radiation are detrimental, low dose radiation (LDR) often protects the organism against a subsequent exposure of lethal doses of radiation. Present study was undertaken to understand the role of *Mre11*, *Rad50* and *Nbs1* genes in the low dose radio-adapted human peripheral blood mononuclear cells (PBMCs). Optimum time interval between low dose (0.07 Gy) and high dose (5.0 Gy) of ^{60}Co - γ -radiation was observed to be 5.0 hours, at which PBMCs showed maximum LDR induced resistance (RIR). At cytogenetic level, micronuclei frequency was found to be reduced in LDR pre-irradiated PBMCs subsequently exposed to high dose radiation (HDR) as compared to controls. At transcriptional level, with reference to sham-irradiated cells significantly ($p \leq 0.05$) altered expression of *Mre11*, *Rad50* and *Nbs1* genes was observed in low dose irradiated cells. At protein level, Mre11, Rad50 and Nbs1 were enhanced significantly ($p \leq 0.05$) in low dose pre-irradiated cells subsequently exposed to high dose of radiation as compared to only high dose irradiated cells. Transcriptional as well as translational modulation in the expression of MRN complex components upon low dose irradiation may confer its participation in repair pathways, resulting in induced resistance.

Keywords: ionizing radiation, radioadaptive response, micronuclei, MRN complex, gene expression, immuno-blotting.

INTRODUCTION

The protective effect of LDR has been considered in various types of cell systems such as plants, microbes, mice, rabbit and human (Upton 2001, Kadhim *et al.* 2004, Luckey 2008, Rithidech and Scott 2008). LDR may be useful in identifying unique biomarkers, risk assessment, cancer therapy and screening the astronauts for long-term space missions. There is increasing interest to understand the therapeutic application of LDR in cancer therapy (Wu *et al.* 2008, Tseng *et al.* 2009). It has been proposed

Address correspondence to Sompal Singh, Department of Zoology, M. S. (PG) College, Saharanpur-247 001, India; Phone: +91-132-2661616; Fax: +91-132-2661818; E-mail: somuatar@gmail.com

that LDR may improve the tumor therapy in combination with chemotherapy by the reduction of damage on the immune system and stimulation of antitumor immune reaction (Yu *et al.* 2007). Protection against radiation induced teratogenesis was also observed in low dose pre-irradiated mice (Okazaki *et al.* 2005). There are few studies on the application of LDR in therapy of non cancerous diseases such as diabetes, asthma and infectious diseases (Wang *et al.* 2008; Fang *et al.* 2005; Khaskhely *et al.* 2002).

Exposure of cells to LDR enhanced the metabolic activities of cells such as DNA repair, synthesis of DNA, RNA and protein. These effects consequently protected cells from gene mutation, DNA damage and chromosomal aberrations caused by subsequent exposure to radiation (Wang and Cai 2000). Cellular responses to ionizing radiation are mediated by genes which control complex pathways. Changes in the expression of some genes in response to LDR may occur within a few hours after irradiation (Franco *et al.* 2005, Jin *et al.* 2008). It was suggested that 0.1 Gy irradiation causes change in gene expression involved in protective function in mice (Yin *et al.* 2003). Many genes have been reported that have significant role in radioadaptive response (RIR) but the critical function and regulation of those genes has not been clear till date. Coleman *et al.* (2005) reported that the radioadaptive response appeared to be associated with the differential expression of diverse genes. They proposed that a balance between DNA repair/stress response genes and cellular proliferation/apoptosis genes is the controlling element of radioadaptive response.

The DNA repair is an essential process in all organisms, and requires the coordinated activities of evolutionarily conserved protein assemblies. One of the most critical of these is the Mre11/Rad50/Nbs1 (MRN) complex, which is conserved from bacteriophages to humans and present in all three biological kingdoms (Cromie *et al.* 2001). All three components of MRN complex are encoded by essential genes and hypomorphic mutations in any of these human genes could result in genomic instability syndromes. MRN complex has been linked to many DNA metabolic processes such as homologous recombination (HR), nonhomologous recombination (NHEJ), DNA double strand break (dsb) processing and checkpoint regulation (van den Bosch *et al.* 2003). Homologous and nonhomologous end-joining DNA repair mechanisms are initiated through the concerted recruitment of the Rad50/Mre11/Nbs1 complex and DNA protein kinase to the DNA damage sites (Dasika *et al.* 1999).

Though MRN complex has been studied relatively at higher doses of radiation (Zhong *et al.* 2005; Maser *et al.* 1997), the role of Mre11, Rad50 and Nbs1 in LDR induced RIR (adaptive response) was not evaluated so far. To realize the importance of MRN complex in DNA repair and other signaling pathways, present study was carried out. In this investigation human PBMCs were exposed to LDR and expression of *Mre11*, *Rad50* and

Modification in the expression of Mre11/Rad50/Nbs1 complex

Nbs1 was analysed at mRNA and protein levels. The influence of modulated expression of MRN complex was further correlated with LDR induced resistance.

MATERIALS AND METHODS

Cells and Culture conditions

Venous blood from non-alcoholic, non-smoker healthy human male donors (20-30 years) was drawn into heparinized vacutainers. PBMCs were isolated from heparinized whole peripheral blood utilizing the ficoll Histopaque (Sigma Aldrich Chemicals, USA) procedure. Cell viability was determined with trypan blue. The PBMCs (1×10^6 cells/ml) were suspended in complete RPMI-1640 supplemented with 10% fetal bovine serum, 100 units/ml penicillin sodium salt, 100 µg/ml streptomycin sulphate and 2 mg/ml sodium bicarbonate. Cells were stimulated with phytohemagglutinin (Difco, Germany) @10µg/ml. Micro-culture of cells were maintained in flat-bottomed 96 well culture plates with 5% CO₂ at 37 °C and cell proliferation was determined by the DNA ligand Hoechst 33342 (Merck, Germany) method (Blaheta *et al.* 1991).

Irradiation

⁶⁰Co-γ-irradiator (GC 220, Canada) with dose rate 45 rad/min was used for irradiation. Low dose and high dose of radiation were determined upon irradiation (0-10 Gy) of 22 hours old culture. Low dose (0.07 Gy) was delivered after 22 hours old cultures and high dose (5.0 Gy) was delivered to the pre-irradiated or non pre-irradiated culture as per experimental design.

Micronuclei assay

Micronuclei assay with some modifications was performed according to Fenech (1993). In brief, cytochalasin B (Sigma Aldrich Chemicals, USA) @ 3 µg/ml was added to the human PBMCs culture at 44 hours after culture initiation. Cells (1×10^6) were harvested at 72 hours and washed twice with saline at 300 × g for 10 min. Cell pellet was suspended in 200 µl chilled carnoy solution (methanol: acetic acid, 3:1) and incubated at 4 °C for 2 hours. The fixed cell suspension was laid on the chilled slides. Slides coated with fixed cells were dried overnight and stained with Hoechst 33342 (10 µg/ml) in dark for 30 min. Micronuclei were counted in binucleated cells at λ_{ex} 355 nm and λ_{em} 460 nm. At least 1000 cells per sample were scored at 400× magnification as per criteria described (Fenech 1993; Countryman and Heddle 1976).

RNA extraction and purification

Total RNA was extracted using RNeasy mini kit as per the manufacturer protocol (Qiagen, Germany). Briefly, cells (5×10^6) were harvested and pooled in 15 ml tube and washed once with PBS at $300 \times g$ for 5 min at 4°C . Cell pellet was lysed in 350 μl RLT buffer containing 10% β -mercaptoethanol. Cell lysate was applied on Qias shredder column and centrifuged at $14000 \times g$ for 2 min. for complete homogenization. Homogenized lysate was mixed with equal volume of 70% ethanol for RNA precipitation. The total content was transferred to silica membrane column for adsorption of RNA to membrane and centrifuged at $8000 \times g$ for 15 sec. DNA and proteins were removed by washing once with RW1 at $8000 \times g$ for 15 sec. and twice with RPE buffer at $8000 \times g$ for 2 min. Finally column was centrifuged at $8000 \times g$ for 2 min. and purified RNA was eluted from the column using RNase-free water. RNA samples were diluted in 1 mM EDTA and OD was measured at 260 nm and 280 nm in the UV-visible spectrophotometer (Biomate, UK). RNA samples with purity ratio ($\text{OD}_{260/280}$) in the range of 1.7-2.0 were processed for downstream applications. To check the integrity of RNA samples, 0.5 μg RNA was loaded in 1% agarose gel and electrophoresis was performed in $0.5 \times \text{TAE}$ at 100V.

RT-PCR

RT-PCR was performed using one step RT-PCR kit (Qiagen, Germany) as per the manufacturer instructions. Each reaction volume was 50 μl contained 1 μg total human RNA mixed with 10 μl $5 \times \text{RT-PCR}$ buffer, 2 μl dNTPs and 2 μl enzyme mix. The primers were added at 0.6 μM final concentration. Primers were designed as per protocols by <http://bibiserv.techfak.uni-bielefeld.de/genefisher> website and synthesized from Integrated DNA Technologies, Coralville. All thermal cycler steps were carried out in iCycler (Bio-Rad, USA). Reverse transcription reaction was carried out at 50°C for 30 min for first strand cDNA synthesis and further denatured at 95°C for 15 min. Thirty five cycles of PCR were completed, each cycle comprising of 94°C for 1.5 min (*Rad50*, *Nbs1*), 1.0 min (*Mre11*); 55°C for 1.5 min (*Rad50*, *Nbs1*), 1.0 min. (*Mre11*); 72°C for 2.0 min (*Rad50*, *Nbs1*), 1.5 min (*Mre11*) and final extension at 72°C for 10 min. PCR products were electrophoresed in 1.5% agarose gel for 50 min. at 100V in $0.5 \times \text{TBE}$. Gels were stained with 0.5% ethidium bromide (Sigma Aldrich Chemicals, USA) and photographed in Night Hawk CCD camera.

*Modification in the expression of Mre11/Rad50/Nbs1 complex***Primers**

Gene name	Primer sequences
<i>Rad50</i> forward primer	5'-CTTATACAGGACCAGCAGGAAC-3'
<i>Rad50</i> reverse primer	5'-CCTTTCTGTCGCCCTAATGC-3'
<i>Nbs1</i> forward primer	5'-CCACCATGTCTAGCTACTTG-3'
<i>Nbs1</i> reverse primer	5'-CTTGACTGGAATCCCTTCTTG-3'
<i>Mre11</i> forward primer	5'-CCAGAGAAGCCTCTTGTACG-3'
<i>Mre11</i> reverse primer	5'-TTCCACCTCTTCGACCTCTTC-3'

Protein extraction and estimation

Cells (5×10^6) were harvested and centrifuged at $300 \times g$ for 10 min. Cell pellet was resuspended in ice cold 0.2 mM phosphate buffer (pH 7.4) containing protease inhibitors (0.1 mM EDTA, 1.0 mM PMSF, 1.0 mM DDT, 0.1 mM EGTA, 0.3% NP-40 and 1.0 $\mu\text{g}/\text{ml}$ pepstatin A) and incubated for 1.0 hour at 4 °C. The cell suspension was sonicated in chilled PBS buffer for 10 sec. at 50 Hz and centrifuged at $10000 \times g$ for 10 min at 4 °C. Supernatant was separated and stored at 4 °C for further analysis of protein. Total soluble protein content was estimated by Bradford method using BSA as the standard.

SDS-PAGE

Protein samples were analysed in 10% separating and 4% stacking poly-acrylamide gels of 0.75 mm thickness as per procedure by Kumar *et al.* (2005). Briefly, equal amount of protein samples (10 μg) were mixed with 2 \times loading buffer and heated in a boiling water bath for 2-3 min. After well mixing, samples were loaded in each well. The acrylamide gel loaded with protein samples was transferred in the assembly (Bio-Rad, USA) and 1 \times electrophoresis buffer was filled. Electrophoresis was carried out at constant 100 V for 1.5 hours. After electrophoresis, gels were carefully separated from the gel plates and stained in 0.1% coomassie brilliant blue R-250 with gentle shaking for 4.0 hours at room temperature. Gels were then destained in destaining solution for 2.0 hours to obtain the distinct bands over the clear background and stored in 1% acetic acid in water for future analysis.

Transfer of proteins and blocking

For western blotting, proteins were transferred on nitrocellulose membrane electrophoretically as per the protocol described by Kumar *et al.* (2005). Briefly, gels containing protein bands were placed between pre-soaked blotting pads facing one side nitrocellulose membrane. This set was packed in cassettes and adjusted in a mini-trans blot assembly (Bio-Rad, USA). Chilled blotting buffer was filled in the assembly con-

taining ice packet and protein bands were transferred at constant voltage (100V) for 1 hour. The nitrocellulose membrane containing protein bands was blocked in blotting solution (5% w/v low fat milk in TBS) for 2.0 hours at room temperature. After blocking, membranes were washed thrice with washing buffer (TBS, 2% Tween 20).

Antibodies probing and detection

Protein expression was analysed by probing with respective primary antibodies specific for human Nbs1 (sc-8580), Mre11 (sc-5859) and Rad50 (sc-18291) from Santa Cruz Biotech., Germany. Primary antibodies were added to the nitrocellulose membrane at 1:1000 dilution in TBS containing 2% Tween 20 and incubated for 2.0 hours with mild shaking at room temperature. Following three washes (20 min. each) in washing buffer (TBS, 2% Tween 20), membranes were incubated with the TBS containing 1:1000 dilution of bovine anti-goat alkaline phosphatase conjugated secondary antibodies (sc-2381, Santa Cruz Biotech., Germany) for 1.0 hour in shaking condition at room temperature. The membranes were again washed three times (20 min. each) with washing buffer and then treated with BCIP-NBT reagent (Sigma Aldrich Chemicals, USA) in dark for 10-30 min. The developed protein blots were further subjected to densitometric analysis. 1D protein Densitometric Analysis Software (AIFA Ease FC) containing automatic inbuilt BSA standard was used for densitometric analysis.

Statistics

The data was presented as mean \pm SD of three separate experiments. The mean values in the experimental design in two treatment comparison were compared using student t's test. In view of evaluation the effect of multiple genes, proteins, time based change in expression levels and concentration parameters, multiple ANOVA (including one-way/two-way ANOVA) analysis was used. Tukey's HSD Test was used as post hoc test for multiple comparisons. Significance was tested and $p < 0.05$ was considered as significant difference in all cases. All the tests were done using SPSS software version-10.

RESULTS

Radiation response of human PBMCs

Proliferation of human PBMCs was found to be varied with different doses of radiation recorded 96 hours after culture initiation (Figure 1). With respect to sham irradiated cells no significant ($p < 0.05$) change in the proliferation of PBMCs irradiated with < 0.10 Gy was observed. On the other hand, cell proliferation was significantly ($p < 0.05$) decreased in the PBMCs which were irradiated with 0.20-10 Gy.

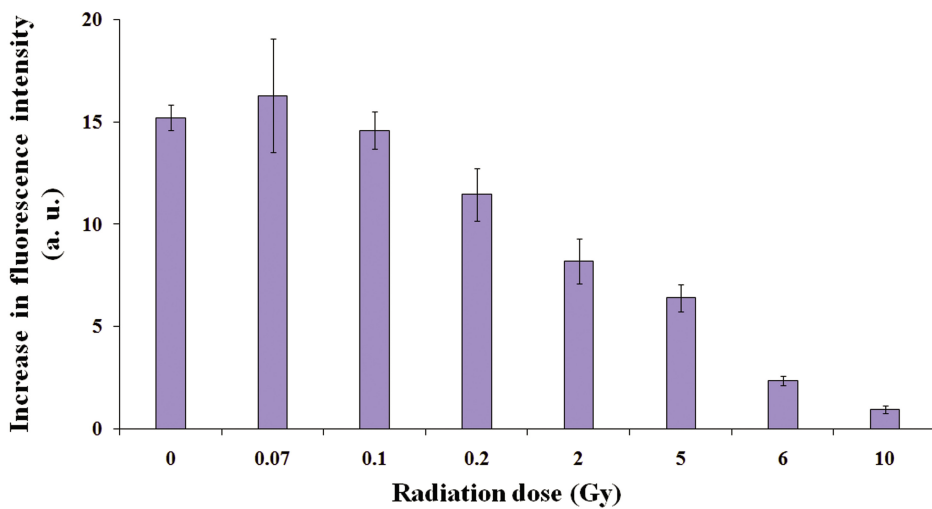
Modification in the expression of Mre11/Rad50/Nbs1 complex

FIGURE 1. Effect of ^{60}Co - γ -rays on human PBMCs proliferation. Increase in fluorescence intensity in PHA stimulated human PBMCs indicates the proliferation. Cells were irradiated at 22 hour after PHA addition and fluorescence was measured at 96 hour old culture. Results were mean \pm SD.

Increase in fluorescence intensity (FI) = $\text{FI}_{\text{stimulated cells}} - \text{FI}_{\text{unstimulated cells}}$

Optimization of time interval between LDR and HDR

Time interval between low dose and HDR is an important factor in the induction of RIR. The different time intervals were studied to obtain the time point where cells showed maximum RIR. No significant ($p < 0.05$) effect of LDR was observed in cell proliferation when time interval between LDR and HDR was < 3.0 hours and > 7.0 hours. Depending upon time duration between LDR and HDR, cell proliferation in low dose irradiated PBMCs subsequently exposed with HDR was enhanced as compared to only high dose irradiated cells. Comparatively, low dose induced resistance in PBMCs was declined upon alteration of time interval from 5.0 hours between LDR and HDR exposure (Figure 2).

Evaluation of micronuclei frequency

Spontaneous micronuclei frequency was varied in different donors between the range 12-18 micronuclei bearing cells per 1000 cells. The effect of 0.07 Gy on the production of micronuclei was slightly varied in all the donors. Induced micronuclei frequency was determined after 5.0 Gy exposure in PBMCs and compared with micronuclei frequency in low dose pre-irradiated cells. The micronuclei frequency induced by high dose of radiation in binucleated cells was found to be reduced in low dose pre-irradiated cells as compared to the only high dose irradiated cells. This reduction in micronuclei frequency in the cells was $23.4 \pm 2.14\%$ - $31.8 \pm 2.29\%$ in different donors (Table 1).

Singh and others

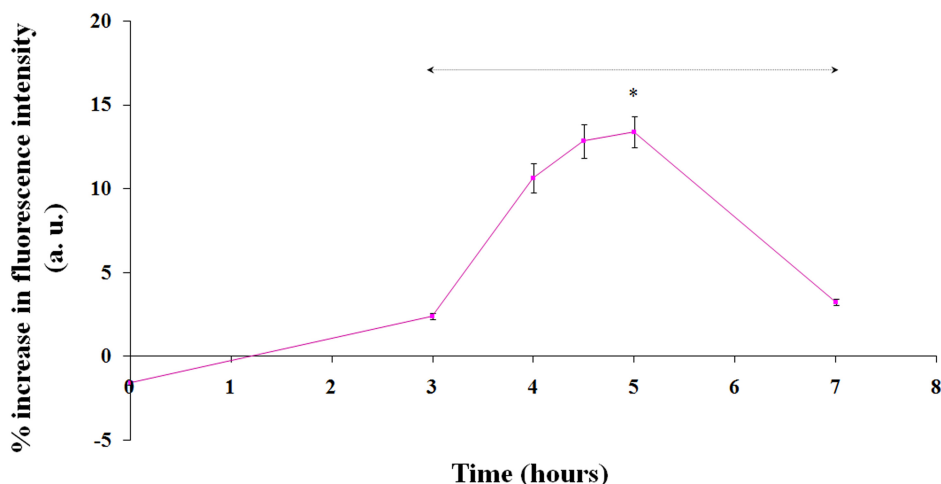


FIGURE 2. Optimization of time intervals between low dose and HDR. Results were mean \pm SD, * indicates maximum % increase in fluorescence intensity at 5.0 hour time interval between low dose and high dose, \leftrightarrow means 3-7 hours time intervals exhibited significant ($p < 0.05$) % increase in the fluorescence intensity. % increase in fluorescence intensity (FI) = $(FI_{\text{LDR+HDR}} - FI_{\text{HDR}}) \times 100 / FI_{\text{HDR}}$.

TABLE 1. Micronuclei (MN) in cultured binucleated human PBMCs at 72 hours.

Donors	No. of micronuclei per 1000 binucleated cells at doses (Gy)				% Micronuclei decrease in low dose pretreated cells
	0	0.07	5.0	0.07+5.0	
I	13 \pm 0.33	15 \pm 1.02	245 \pm 11.08	167 \pm 8.92*	31.8 \pm 2.29
II	18 \pm 0.89	16 \pm 1.26	166 \pm 9.67	89 \pm 6.65*	23.4 \pm 2.14
X	12 \pm 0.65	14 \pm 0.88	298 \pm 15.38	206 \pm 8.77*	30.8 \pm 2.47

$$\% \text{ decrease in MN} = (MN_{\text{HDR}} - MN_{\text{LDR+HDR}}) \times 100 / MN_{\text{HDR}}$$

Cells were stained with Hoechst 33342 and total 1000 binucleated cells were scored per sample at 400 \times magnification in fluorescence microscope.

* indicates significant ($p < 0.05$) decrease in the number of micronuclei per 1000 cells in low dose and high dose irradiated cells with respect to their respective controls (5.0 Gy irradiated cells).

Transcriptional expression of MRN complex after irradiation

The transcriptional expression of *Mre11*, *Rad50* and *Nbs1* genes in low dose (0.07 Gy) irradiated human PBMCs is shown in Figure 3. *Rad50* was induced after low dose irradiation in PBMCs. A significant ($p < 0.05$) increase (1.2 fold) in the expression of *Rad50* mRNA (Lane 3) was observed in low dose irradiated cells as compared to sham irradiated control (Lane 2). Similarly, expression level of *Mre11* was also found to be enhanced (1.46 fold) in low dose irradiated cells (Lane 7) as compared to non irradiated PBMCs (Lane 6). On the contrary, mRNA level of *Nbs1* was drastically reduced 5.0 hours after low dose irradiation. This reduc-

Modification in the expression of Mre11/Rad50/Nbs1 complex

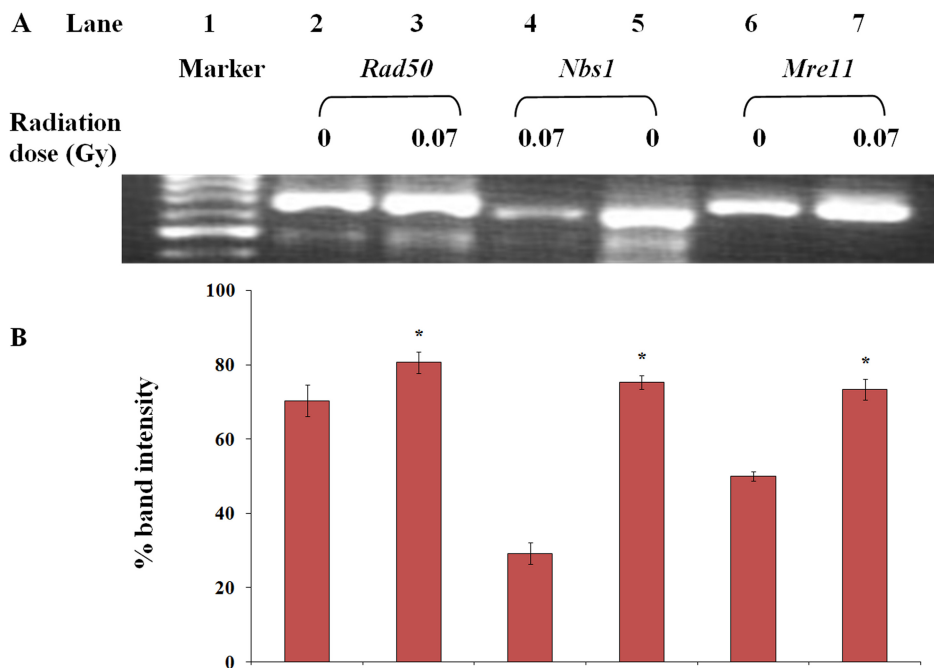


FIGURE 3. Transcriptional expression of *Rad50*, *Nbs1*, *Mre11* genes. Human PBMCs were stimulated with PHA and 22 hours old culture was irradiated with low dose (0.07 Gy) of ^{60}Co - γ -rays. Total RNA was extracted after 5.0 hours of irradiation. 100 bp DNA ladder from MBI Fermentas was used as a marker. A. Transcriptional level of *Rad50*, *Nbs1*, *Mre11* genes. B. Quantitative densitometric analysis of expression pattern. Results were mean \pm SD, * indicates significant ($p < 0.05$) increase/decrease in the intensity of gene i.e. *Rad50*, *Nbs1*, *Mre11* bands with respect to sham irradiated respective control groups.

tion in *Nbs1* mRNA in low dose irradiated cells (Lane 4) was 2.58 fold as compared to the sham irradiated cells (Lane 5).

Evaluation of radiation effect on MRN complex protein expression

Expression of MRN complex was also evaluated at protein level. To find out the protein level of Mre11, Rad50 and Nbs1 after low dose irradiation, human PBMCs were irradiated with LDR and protein level was studied using immuno-blotting (Figure 4). With reference to sham irradiated control (Lane 1) Mre11 protein level was not observably changed in PBMCs after 2.5 hours of LDR exposure (Lane 2). However, the protein level of Mre11 was enhanced (1.28 fold) after 5.0 hours of low dose irradiation (Lane 4) as compared to sham irradiated cells (Lane 3). Specifically, the Mre11 protein was comparatively 1.22 fold elevated in LDR exposed PBMCs subsequently challenged with HDR (Lane 6) as compared to controls (Lane 5).

Similarly, protein level of Rad50 was not significantly ($p < 0.05$) changed until 2.5 hours after low dose irradiation (Lane 2) as compared

Singh and others

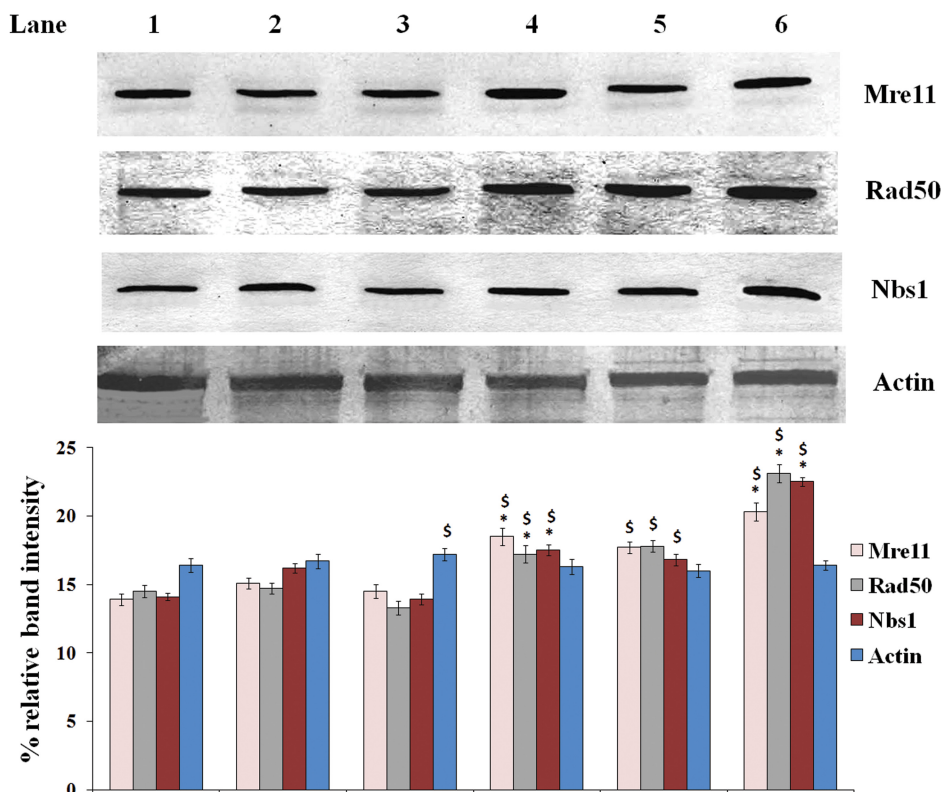


FIGURE 4. LDR effect on Mre11, Rad50 and Nbs1 expression in human PBMCs. Radiation was delivered at 22 hours (low dose, 0.07 Gy) and 27.0 hours (high dose, 5.0 Gy) after PHA addition.

Lane 1: 0 Gy +2.5 h

Lane 2: 0.07 Gy +2.5 h

Lane 3: 0 Gy +5.0 h

Lane 4: 0.07 Gy +5.0 h

Lane 5: 0 Gy +5.0 h +5.0 Gy +2.0 h

Lane 6: 0.07 Gy +5.0 h +5.0 Gy +2.0 h

Actin (2.0 µg) was loaded per lane as a positive control. Densitometric analysis representing % relative intensity of corresponding protein blots. Results were mean \pm SD, * indicates significant ($p < 0.05$) difference in the band intensity with their respective controls. \$ means significant ($p < 0.05$) increase/decrease in the intensity of bands at different time intervals with respect to their sham irradiated groups.

to the sham irradiated PBMCs (Lane 1). However, the protein level was 1.29 fold enhanced 5.0 hours after LDR exposure (Lane 4) than corresponding control (Lane 3). Rad50 protein level in low dose irradiated cells subsequently exposed with HDR was 1.30 fold higher (Lane 6) as compared to the non pre-irradiated PBMCs (Lane 5). In case of Nbs1, enhanced Nbs1 protein was observed in all irradiated samples as compared to their controls. The increase in Nbs1 protein level was observed 1.17 fold (Lane 2) and 1.26 fold (Lane 4) upon 2.5 hours and 5.0 hours after low dose irradiation respectively in PBMCs as compared to their cor-

responding controls. In low dose pre-irradiated cells, the Nbs1 protein level was 1.34 fold higher (Lane 6) as compared to the cells, which were irradiated with only HDR (Lane 5).

DISCUSSION

LDR induced resistance involves protection at the cellular level due to stimulation of induced repair processes, resulting in protection of cells against the inactivating effect of subsequent high dose irradiation. A range of LDR (0.01 Gy to 0.20 Gy) has been reported for human cells which is responsible in the induction of radio-adaptation (Shadley and Wiencke 1989; Matsumoto *et al.* 2004). Since RIR depends on physiological, physical and genetic factors, we determined 0.07 Gy and 5.0 Gy as low and high dose of radiation respectively for cultured human PBMCs (Figure 1). RIR was lasted for 3-7 hours after low dose irradiation in human PBMCs with a peak at 5.0 hours (Figure 2). At cytogenetic level, micronuclei frequency was determined at the time point, where time interval was 5.0 hours between low dose and high dose of radiation. Micronuclei frequency in 0.07 Gy radio-adapted cells was found to be reduced comparatively in the cells which were subsequently exposed to 5.0 Gy (Table 1). Therefore, this may be expected that 0.07 Gy induced the radio-resistance in human PBMCs to subsequent high dose (5.0 Gy) of radiation. The reduction in micronuclei frequency was varied in different donors. These results were consistent with previous findings that radioadaptive response varied among different individuals/cell lines (Correa and Cheung; 2004; Jiang *et al.* 2008).

It has been showed that the transcription and translation of genes associated with DNA repair and cell cycle regulation were required for RIR in human lymphocytes. Working hypothesis suggested that an inducible molecular process triggered by low doses of radiation leads to cell protection against the deleterious effects of subsequent high dose irradiation. DNA repair has been suggested to play major role in the LDR induced resistance in microbial, plant and human system (Kadhim *et al.* 2004; Sasaki *et al.* 2002). The components of nucleotide excision repair pathway have been reported to play role in adaptive response by 0.05 Gy in mammalian cells (Hafer *et al.* 2007). Participation of HR in *S. cerevisiae* whereas NHEJ in human has been proposed for the induction of adaptive response (Sasaki *et al.* 2002). Expression of various genes involved in DNA repair, cell cycle arrest, immune system and signaling pathways has been studied after LDR exposure in various cell types (Coleman *et al.* 2005; Goldberg *et al.* 2004; Berglund *et al.* 2008; Shigematsu *et al.* 2007, Fachin *et al.* 2009). However, no complete pathway is confirmed in the participation of LDR induced protection. DNA repair genes i.e. *Mre11*, *Rad50* and *Nbs1* serve as initial factor and have been reported to participate both in NHEJ and HR pathway (Rapp and Greulich 2004; Jackson 2002). The

role of MRN complex has been extensively studied at relatively higher doses of radiation but the studies at LDR are inadequate. Therefore, expression of *Mre11*, *Rad50* and *Nbs1* and their products was evaluated after low dose irradiation in human lymphocytes.

Mre11 is the core of MRN complex. This multifunctional protein interacts independently with both Nbs1 and Rad50 (Iijima *et al.* 2008). It processes the DNA double strand breaks through 3'→5' dsDNA exonuclease, ssDNA and dsDNA endonuclease activities (Assenmacher and Hopfner 2004). The other component of MRN complex i.e Rad50 tethers the broken DNA ends and keeps them in close proximity (van den Linden *et al.* 2009). Recently, the role of Rad50 has been suggested in RIR using *S. cerevisiae* (Bala and Goel 2007). Elevated expression of Rad50 in low dose (0.50 Gy) irradiated human lymphocytes has also been reported (Fachin *et al.* 2007). But no report demonstrated the expression of Mre11 and Rad50 in radio-adapted human lymphocytes. Though both these genes differed in their functions in the DNA repair process, we observed that they elicit similar pattern of expression at transcription and protein level (Figure 3 & 4). Transcripts level of *Mre11* and *Rad50* was elevated in low dose radio-adapted human PBMCs which may suggest any possibility of these genes in LDR response. Time dependent expression also exhibited variation in the protein level in these irradiated cells. While there was no remarkable change in the Mre11 and Rad50 level until 2.5 hours but thereafter it was enhanced and remained elevated even after exposure to challenge dose of radiation as compared to their controls (Figure 4). So it appears that both these genes respond to LDR beyond 2.5 hours of irradiation which fetches attention towards the induction of RIR in human PBMCs that is apparent beyond 3.0 hours of LDR exposure (Figure 2). Although RIR is a complex process involving various molecules and there pathways, the possible role of Mre11 and Rad50 in the phenomenon of RIR could not be turned down.

The third component (Nbs1) of MRN complex is involved in the recruitment of this complex at DNA damaged sites. In case of Nbs1 deficient cells, MR complex was not observed at DNA damaged sites, rather it was confined to the cytoplasm after irradiation (Tauchi *et al.* 2001). In contrast to elevated expression of *Mre11* and *Rad50*, the mRNA level of *Nbs1* was repressed after low dose irradiation as compared to sham irradiated samples (Figure 3). However, the Nbs1 protein level was enhanced at 2.5 hours after low dose irradiation, but Mre11 and Rad50 expression was constitutive up to 2.5 hours after LDR exposure (Figure 4). These observations suggested that Nbs1 is the early responder of irradiation. Moreover, Nbs1 contains binding domain for Mre11 to form the MRN complex and binds with γ -H2AX protein through its FHA/BRCT domain at the DNA dsb sites to recruit the complex (Kobayashi 2004). γ -H2AX co-localizes with the foci of MRN complex within 2.0 hours after irradiation

Modification in the expression of Mre11/Rad50/Nbs1 complex

(Kobayashi *et al.* 2002). Therefore, it may be inferred that the constitutive level of MR complex is carried by the newly formed Nbs1. It is also known that radio-adaptation is a transient phenomenon which lasts for a short window of time and second exposure of LDR is needed to maintain the RIR (Matsumoto *et al.* 2004). It might be expected that further induction of Nbs1 may require second exposure of LDR to human PBMCs. Thus these observations favor the participation of Nbs1 in RIR phenomenon.

MRN complex plays as initiator in HR and NHEJ pathways. Therefore, keeping in mind the importance of this complex, enhanced level of Mre11, Rad50 and Nbs1 in low dose radio-adapted human PBMCs indicates the role of MRN complex in RIR. But the mechanism adopted by this complex to regulate the cells in radio-protection has to be identified. MRN complex is an integral part of DNA repair and associated with genetic disorders, therefore our study strongly suggests the possible induction of DNA repair by this complex in radio-adapted human PBMCs.

ACKNOWLEDGEMENTS

Authors are extremely thankful for the UGC in providing fellowship and DRDO for funding the research throughout this work. Dr. RP Tripathi, Director, INMAS, Delhi is also acknowledged for providing research facility.

REFERENCES

- Assenmacher N and Hopfner KP. 2004. MRE11/RAD50/NBS1: complex activities. *Chromosoma* 113: 157-166.
- Bala M and Goel HC. 2007. Modification of low dose radiation induced radioresistance by 2-deoxy-D-glucose in *Saccharomyces cerevisiae*: mechanistic aspects. *J Radiat Res* 48: 335-346.
- Berglund SR, Rocke DM, Dai J, Schwietert CW, Santana A, Stern RL, Lehmann J, Hartmann Siantar CL and Goldberg Z. 2008. Transient genome-wide transcriptional response to low-dose ionizing radiation in vivo in humans. *Int J Radiat Oncol Biol Phys* 70: 229-234.
- Blaheta RA, Franz M, Auth MK, Wenisch HJ and Markus BH. 1991. A rapid non-radioactive fluorescence assay for the measurement of both cell number and proliferation. *J Immunol Methods* 142: 199-206.
- Coleman MA, Yin E, Peterson LE, Nelson D, Sorensen K, Tucker JD and Wyrobek AJ. 2005. Low-dose irradiation alters the transcript profiles of human lymphoblastoid cells including genes associated with cytogenetic radioadaptive response. *Radiat Res* 164: 369-382.
- Correa CR and Cheung VG. 2004. Genetic variation in radiation-induced expression phenotypes. *Am J Hum Genet* 75: 885-890.
- Countryman PI and Heddle JA. 1976. The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. *Mutat Res* 41: 321-332.
- Cromie GA, Connelly JC and Leach DR. 2001. Recombination at double-strand breaks and DNA ends: conserved mechanisms from phage to humans. *Mol Cell* 8: 1163-1174.
- Dasika GK, Lin SC, Zhao S, Sung P, Tomkinson A and Lee EY. 1999. DNA damage-induced cell cycle checkpoints and DNA strand break repair in development and tumorigenesis. *Oncogene* 18: 7883-7899.
- Fachin AL, Mello SS, Sandrin-Garcia P, Junta CM, Donadi EA, Passos GA and Sakamoto-Hojo ET. 2007. Gene expression profiles in human lymphocytes irradiated in vitro with low doses of gamma rays. *Radiat Res* 168: 650-665.

- Fachin AL, Mello SS, Sandrin-Garcia P, Junta CM, Ghilardi-Netto T, Donadi EA, Passos GA and Sakamoto-Hojo ET. 2009. Gene expression profiles in radiation workers occupationally exposed to ionizing radiation. *J Radiat Res* 50: 61-71.
- Fang SP, Tago F, Tanaka T, Simura N, Muto Y, Goto R and Kojima S. 2005. Repeated irradiations with gamma-rays at a Dose of 0.5 Gy may exacerbate asthma. *J Radiat Res* 46: 151-156.
- Fenech M. 1993. The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations. *Mutat Res* 285: 35-44.
- Franco N, Lamartine J, Frouin V, Le Minter P, Petat C, Leplat JJ, Libert F, Gidrol X and Martin MT. 2005. Low-dose exposure to gamma rays induces specific gene regulations in normal human keratinocytes. *Radiat Res* 163: 623-635.
- Goldberg Z, Schwieter CW, Lehnert B, Stern R and Nami I. 2004. Effects of low-dose ionizing radiation on gene expression in human skin biopsies. *Int J Radiat Oncol Biol Phys* 58: 567-574.
- Hafer K, Iwamoto KS, Scuric Z and Schiestl RH. 2007. Adaptive response to gamma radiation in mammalian cells proficient and deficient in components of nucleotide excision repair. *Radiat Res* 168: 168-174.
- Iijima K, Ohara M, Seki R and Tauchi H. 2008. Dancing on damaged chromatin: functions of ATM and the RAD50/MRE11/NBS1 complex in cellular responses to DNA damage. *J Radiat Res* 49: 451-464.
- Jackson SP. 2002. Sensing and repairing DNA double-strand breaks. *Carcinogenesis* 23: 687-696.
- Jiang H, Li W, Li X, Cai L and Wang G. 2008. Low-dose radiation induces adaptive response in normal cells, but not in tumor cells: *in vitro* and *in vivo* studies. *J Radiat Res* 49: 219-230.
- Jin YW, Na YJ, Lee YJ, An S, Lee JE, Jung M, Kim H, Nam SY, Kim CS, Yang KH, Kim SU, Kim WK, Park WY, Yoo KY, Kim CS and Kim JH. 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.
- Kadhim MA, Moore SR and Goodwin EH. 2004. Interrelationships amongst radiation-induced genomic instability, bystander effects, and the adaptive response. *Mutat Res* 568: 21-32.
- Khaskhely NM, Maruno M, Uezato H, Takamiyagi A, Ramzi ST, Al-Kasem KM, Kariya K, Toda T, Hashiguchi Y, Gomez Landires EA and Nonaka S. 2002. Low-dose UVB contributes to host resistance against *Leishmania amazonensis* infection in mice through induction of gamma interferon and tumor necrosis factor alpha cytokines. *Clin Diagn Lab Immunol* 9: 677-686.
- Kobayashi J, Tauchi H, Sakamoto S, Nakamura A, Morishima K, Matsuura S, Kobayashi T, Tamai K, Tanimoto K and Komatsu K. 2002. NBS1 localizes to gamma-H2AX foci through interaction with the FHA/BRCT domain. *Curr Biol* 12: 1846-1851.
- Kobayashi J. 2004. Molecular mechanism of the recruitment of NBS1/hMRE11/hRAD50 complex to DNA double-strand breaks: NBS1 binds to gamma-H2AX through FHA/BRCT domain. *J Radiat Res* 45: 473-478.
- Kumar R, Singh PK, Arora R, Sharma A, Prasad J, Sagar R, Singh S and Sharama RK. 2005. Radioprotection by *Podophyllum hexandrum* in the liver of mice: A mechanistic approach. *Environ Toxicol Pharmacol* 20: 326-334.
- Luckey TD. 2008. Atomic bomb health benefits. *Dose Response* 6: 369-382.
- Maser RS, Monsen KJ, Nelms BE and Petrini JH. 1997. hMre11 and hRad50 nuclear foci are induced during the normal cellular response to DNA double-strand breaks. *Mol Cell Biol* 17: 6087-6096.
- Matsumoto H, Takahashi A and Ohnishi T. 2004. Radiation-induced adaptive responses and bystander effects. *Biol Sci Space* 18: 247-254.
- Okazaki R, Ootsuyama A and Norimura T. 2005. Radioadaptive response for protection against radiation-induced teratogenesis. *Radiat Res* 163: 266-270.
- Rapp A and Greulich KO. 2004. After double-strand break induction by UV-A, homologous recombination and nonhomologous end joining cooperate at the same DSB if both systems are available. *J Cell Sci* 117: 4935-4945.
- Rithidech KN and Scott BR. 2008. Evidence for Radiation Hormesis After In Vitro Exposure of Human Lymphocytes to Low Doses of Ionizing Radiation section sign. *Dose Response* 6: 252-271.
- Sasaki MS, Ejima Y, Tachibana A, Yamada T, Ishizaki K, Shimizu T and Nomura T. 2002. DNA damage response pathway in radioadaptive response. *Mutat Res* 504: 101-118.
- Shadley JD and Wiencke JK. 1989. Induction of the adaptive response by X-rays is dependent on radiation intensity. *Int J Radiat Biol* 56: 107-118.

Modification in the expression of Mre11/Rad50/Nbs1 complex

- Shigematsu A, Adachi Y, Koike-Kiriyama N, Suzuki Y, Iwasaki M, Koike Y, Nakano K, Mukaide H, Imamura M and Ikehara. 2007. Effects of low-dose irradiation on enhancement of immunity by dendritic cells. *J Radiat Res* 48: 51-55.
- Tauchi H, Komatsu K, Mizutani S, Micciché F, Masnada D, Nakanishi M, Tauchi H, Komatsu K, Mizutani S, Khanna K, Chen P, Concannon P, Chessa L and Delia D. 2001. Chk2 activation dependence on Nbs1 after DNA damage. *Mol Cell Biol* 21: 5214-5222.
- Tseng CW, Trimble C, Zeng Q, Monie A, Alvarez RD, Huh WK, Hoory T, Wang MC, Hung CF and Wu TC. 2009. Low-dose radiation enhances therapeutic HPV DNA vaccination in tumor-bearing hosts. *Cancer Immunol Immunother* 58: 737-748.
- Upton AC. 2001. Radiation hormesis: Data and interpretations. *Crit Rev Toxicol* 31: 681-695.
- van den Bosch M, Bree RT and Lowndes NF. 2003. The MRN complex: coordinating and mediating the response to broken chromosomes. *EMBO Rep* 4: 844-849.
- van der Linden E, Sanchez H, Kinoshita E, Kanaar R and Wyman C. 2009. RAD50 and NBS1 form a stable complex functional in DNA binding and tethering. *Nucleic Acids Res* 37: 1580-1588.
- Wang GJ and Cai L. 2000. Induction of cell-proliferation hormesis and cell-survival adaptive response in mouse hematopoietic cells by whole-body low-dose radiation. *Toxicol Sci* 53: 369-376.
- Wang GJ, Li XK, Sakai K and Lu Cai. 2008. Low-dose radiation and its clinical implications: diabetes. *Hum Exp Toxicol* 27: 135-142.
- Wu N, Jin SZ, Pan XN and Liu SZ. 2008. Increase in efficacy of cancer radiotherapy by combination with whole-body low dose irradiation. *Int J Radiat Biol* 84: 201-210.
- Yin E, Nelson DO, Coleman MA, Peterson LE and Wyrobek AJ. 2003. Gene expression changes in mouse brain after exposure to low-dose ionizing radiation. *Int J Radiat Biol* 79: 759-775.
- Yu HS, Xue HW, Guo CB, Song AQ, Shen FZ, Liang J and Deng C. 2007. Low dose radiation increased the therapeutic efficacy of cyclophosphamide on S(180) sarcoma bearing mice. *J Radiat Res* 48: 281-288.
- Zhong H, Bryson A, Eckersdorff M and Ferguson DO. 2005. Rad50 depletion impacts upon ATR-dependent DNA damage responses. *Hum Mol Genet* 14: 2685-2693.