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

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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-$\gamma$-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
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 (1×10⁶ 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 (1×10⁶) 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 λₜₕ 355 nm and λₑₘ 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 (5×10⁶) were harvested and pooled in 15 ml tube and washed once with PBS at 300 × 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 Qiashredder column and centrifuged at 14000 × 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 × g for 15 sec. DNA and proteins were removed by washing once with RW1 at 8000 × g for 15 sec. and twice with RPE buffer at 8000 × g for 2 min. Finally column was centrifuged at 8000 × 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 (OD₂₆₀/₂₈₀) 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 0.5x 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 × 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 0.5x TBE. Gels were stained with 0.5% ethidium bromide (Sigma Aldrich Chemicals, USA) and photographed in Night Hawk CCD camera.
**Primers**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Primer sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rad50 forward primer</td>
<td>5'-CTTATACAGGACCAGCAGGAAC-3'</td>
</tr>
<tr>
<td>Rad50 reverse primer</td>
<td>5'-CCTTTCTGTCGCCCTAATGC-3'</td>
</tr>
<tr>
<td>Nbs1 forward primer</td>
<td>5'-CCACCATTGTCTAGCTACTTG-3'</td>
</tr>
<tr>
<td>Nbs1 reverse primer</td>
<td>5'-CTTGACTGGAACTCCCTTCTTG-3'</td>
</tr>
<tr>
<td>Mre11 forward primer</td>
<td>5'-CCAGAGAAGGCTCTTGTACG-3'</td>
</tr>
<tr>
<td>Mre11 reverse primer</td>
<td>5'-TTCCACCTGTTGACCTGTTG-3'</td>
</tr>
</tbody>
</table>

**Protein extraction and estimation**

Cells (5×10⁶) were harvested and centrifuged at 300 × 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 µg/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 × 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× 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× 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-socked 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-
containing 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 ±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.
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±2.14%-31.8±2.29% in different donors (Table 1).
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-

\[ \text{% decrease in MN} = \left( \text{MN}_{\text{HDR}} - \text{MN}_{\text{LDR-HDR}} \right) \times 100 / \text{MN}_{\text{HDR}} \]

Cells were stained with Hoechst 33342 and total 1000 binucleated cells were scored per sample at 400× 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).

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

<table>
<thead>
<tr>
<th>Donors</th>
<th>No. of micronuclei per 1000 binucleated cells at doses (Gy)</th>
<th>% Micronuclei decrease in low dose pretreated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.07</td>
</tr>
<tr>
<td>I</td>
<td>15±0.33</td>
<td>15±1.02</td>
</tr>
<tr>
<td>II</td>
<td>18±0.89</td>
<td>16±1.26</td>
</tr>
<tr>
<td>X</td>
<td>12±0.65</td>
<td>14±0.88</td>
</tr>
</tbody>
</table>

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

FIGURE 2. Optimization of time intervals between low dose and HDR. Results were mean ±SD. * indicates maximum % increase in fluorescence intensity at 5.0 hour time interval between low dose and high dose, ↔ means 3-7 hours time intervals exhibited significant \((p<0.05)\) % increase in the fluorescence intensity. % increase in fluorescence intensity \((FI) = (FI_{LDR-HDR} - FI_{HDR}) \times 100 / FI_{HDR}\).
Modification in the expression of Mre11/Rad50/Nbs1 complex

A Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7
--- | --- | --- | --- | --- | --- | --- | ---
Marker | Rad50 | Nbs1 | Mre11
Radiation dose (Gy) | 0 | 0.07 | 0.07 | 0 | 0.07 | 0.07

| 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 60Co-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 ±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.

Expression 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
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-

**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 ±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.
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).
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 exonu-
clease, ssDNA and dsDNA endonuclease activities (Assenmacher and
Hopfner 2004). The other component of MRN complex i.e *Rad50* teth-
ers 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 exhib-
it 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 irra-
diation 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* de-
cicient 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 irra-
diated 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.
(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.

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REFERENCES


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