5.3 HIGH LET RADIATION BIOLOGY RESEARCH AT NSC

A. Sarma

The normal experiments conducted in this field involve chromosome aberration and activation of signalling molecules due to charged particle interaction with mammalian cells. Research group from Bhabha Atomic Research Centre, Mumbai, has used ^{16}O and ^{12}C beam on V79 cell line to study a few signalling molecules. In future some studies on mutation as well as radiation protection are being planned.

5.3.1 Expression of NF-κB and ERK following Heavy Ion Irradiation

A.K. Mitra[1], A. Sarma[2], M. Krishna[1] and N.C. Verma[1]

[1]Radiation Biology Division, Bhabha Atomic Research Centre, Trombay
[2]Nuclear Science Centre, New Delhi-110 067

Introduction

Exposure of mammalian cells to ionising radiation results in the activation of a number of existing cellular response pathways. These include the various kinases (1-3). Prominent among them are the stress activated, proapoptotic SAP/JUN kinases (2) and the antiapoptotic NFκB (3). The cell cycle is simultaneously arrested due to activation of DNA damage response proteins like ATM, poly(ADP ribose) polymerase (PARP) and p53 (4). Radiation induced cell cycle delays have been found to be dependent on the quality of radiation i.e. the Linear Energy Transfer (LET). It has been demonstrated that high LET radiation produces more profound G2/M block per unit dose than low LET radiation (5). Such difference in cell cycle delays could be traced back to the DNA damage response proteins, enzymes that get activated following DNA strand breaks. Moreover, differential gene expression has also been reported following low doses of high or low LET radiation (6). Small doses of high LET radiation is also known to increase the radioresistance of Chinese hamster V79 cells (7).

Low and high LET radiation differ in the type of damage, the latter resulting in a cluster of damages and persistent double strand breaks (dsb) (8). These unrepaired dsbs may lead to a signaling pattern that is very different from low LET radiation. The following investigation has been undertaken to investigate whether heavy ion irradiation leads to the activation of cytoprotective signaling factors (NF-κB, ERK etc.) and whether the pattern of activation is the same as low LET (10).

Materials and methods

Cell Culture: Chinese hamster V79 cells were cultured in 75 cm^2 plastic flasks in alpha MEM (Hyclone, USA.) supplemented with 10% foetal calf serum (Sigma, USA.) and kept at 37^0C in humidified atmosphere with 5% CO_2.

Irradiation: Heavy ion irradiation ($^{16}O^{7+}$) was carried out using Radiation Biology beam line of 16 MV 15 UD Pelletron at Nuclear science centre, New Delhi. The primary heavy ion beam from the Pelletron was diffused using a gold foil and low flux beam was obtained at the exit window made of 6.25 mg/cm^2 thick aluminium foil. Specially fabricated stainless steel rings (2.5 cm diameter) were used as petri plates for sample irradiation. A 6 μm thick polypropylene film was attached to the ring to form the base on which 0.5 x 10^6 cells were seeded 24 h before irradiation. Immediately before irradiation, the medium was drained leaving a thin film of medium on the monolayer of cells attached to the polypropylene. The cells were irradiated under sterile conditions, at atmospheric pressure and were exposed to the ions through the polypropylene film. Fresh medium was added following irradiation and cells were kept back in the CO_2 incubator for different periods of time (up to 4 h) before they were lysed.

Western Blotting: Cells (2 X $10^{6)}$ were lysed in 150 μl of 1 x SDS gel-loading buffer (50mMTris.Cl, pH 6.8; 100 mM dithiothreitol; 2% SDS; 0.1 % bromophenol blue and 20% glycerol). The lysate (10 μl) was run on 8% SDS Polyacrylamide Gel followed by transfer to Nitrocellulose membrane (Amersham, USA). Antibodies against NFκB and ERK (Cat. Nos. N67620 and E17120, Transduction Laboratories, USA) were used at dilution of 1:1000 whereas anti-β-Actin (Cat. No. 691001, ICN, USA) was used at a dilution of 1:80,000. The membranes were then probed with horseradish peroxidase conjugated secondary antibody against mouse/rabbit (Roche Molecular Biochemicals, Germany) at a dilution of 1:2000 and developed using BM Chemiluminiscence Western Blotting Kit (Roche Molecular Biochemicals, Germany). Densitometry was done using Shimadzu CS 9000 Dual wavelength flying spot scanner.

Results

Nuclear factor κB (NFκB), a transcription factor available in the cytoplasm is a p50 and p65 heterodimer complexed to a regulatory, inactive subunit I-κB. The dissociation of the heterodimer from I-κB and its translocation to the nucleus can be brought by ionising radiation (11). In the present study, p65 shows a decrease in expression at 30 min. followed by a progressive increase at 2 h and 4 h (Fig. 1). At 4 h it was as much as the control.

Pan ERK, which shows binding at 90 kD and 44 kD, showed a distinctively different expression pattern from PARP and NFκB. ERK1 showed a marginal increase at 30 min. followed by a decrease at 2 h and then an increase at 4 h (Fig. 2). MAP Kinase (90 kD band) showed a significant decrease at 30 min, the level declining to a minimal at 2 h followed by a drastic increase at 4 h (Fig. 3). This MAP kinase is probably the MEKK1, a

126 kD protein that is cleaved by caspases to a 90 kD fragment which then activates apoptosis (12).

Discussion and conclusions

The expression of NFκB exhibits a drop at 30 min followed by an increase in expression at 2 h and 4 h. NFκB is known to induce manganese superoxide dismutase (13), an antioxidant scavenging enzyme, that helps in reducing radiation induced damage. Besides acting as a redox regulator, activation of NFκB by TNF or ionising radiation provides protection against apoptotic cell death induced by these stimuli (14).

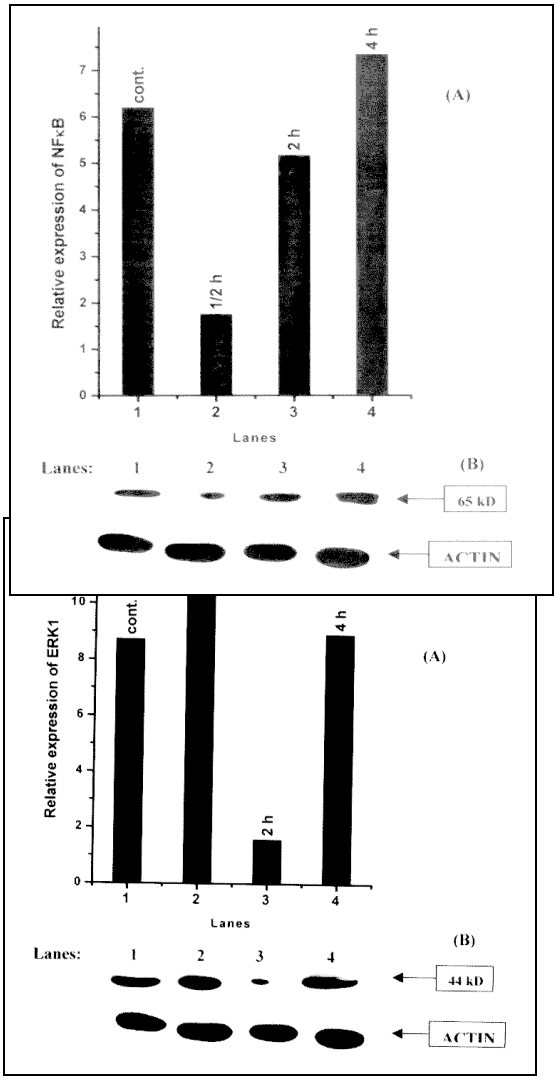


Fig. 1 : Time course of NF-κB (p65) expression following heavy ion irradiation. V79 cells were exposed to 1 Gy dose of $^{16}O^{7+}$ beam and lysed ½ h, 2 h and 4 h post-irradiation. Lysates were subjected to western blotting studies. (A) Blot analysis and (B) Western Blot

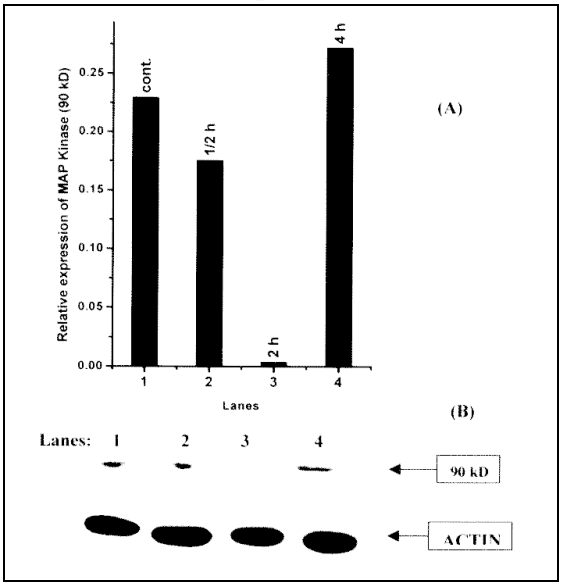
Key: *Lane 1*: Unirradiated; *Lane 2*: ½ h post-irradiation; *Lane 3*: 2 h post-irradiation; *Lane 4*: 4 h post-irradiation.

Fig. 2 : Time course of ERK1 expression following heavy ion irradiation. V79 cells were exposed to 1 Gy dose of $^{16}O^{7+}$ beam and lysed ½ h, 2 h and 4 h post-irradiation. Lysates were subjected to western blotting studies. (A) Blot analysis and (B) Western Blot for ERK1.

Key: *Lane 1*: Unirradiated; *Lane 2*: ½ h post-irradiation; *Lane 3*: 2 h post-irradiation; *Lane 4*: 4 h post-irradiation.

Fig. 3 : Time course of 90 kD MAP kinase expression following heavy ion irradiation. V79 cells were exposed to 1 Gy dose of $^{16}O^{7+}$ beam and lysed ½ h, 2 h and 4 h post-irradiation. Lysates were subjected to western blotting studies. (A) Blot analysis and (B) Western Blot for MAP Kinase (90 kD).

Key: *Lane 1*: Unirradiated; *Lane 2*: ½ h post-irradiation; *Lane 3*: 2 h post-irradiation; *Lane 4*: 4 h post-irradiation.



The expression of ERK1 was significantly increased at 30 min and 4 h. This belongs to the cytoprotective cascade that is activated following radiation. The regulatory function of MAP Kinase in cell proliferation is known to be dependent on the magnitude and duration of its activation. Although MAP Kinase pathway is generally regarded to be strongly responsive to mitogenic signals, the only stressful signal that strongly activates MAP Kinase is ionising radiation (16). It has now become clear that the pathway is also essential for progression through G_2/M checkpoint, the checkpoint at which the cell cycle is arrested following ionising radiation.

Between the time of irradiation to 4 h, it seems that there are fluctuations in the expression of NFκB and ERK1 and 90 kD MAP kinase. Fluctuations and biphasic expressions had been reported earlier with p53 and have been attributed to the differential expression of MDM2 (15). No such fluctuations have been reported with NF-κB or ERK. Most often with γ-radiation there is a gradual increase in the expression of the signalling factors followed by a decrease in expression as the time increases. The signaling molecules examined (NF-κB and ERK1) are in some way associated with the radioresistance of a cell. From these results it seems that like γ-radiation the fate of the cell in terms of

apoptosis or survival is dependent on the interplay of the signaling factors. Persistent activation of ERK1 and/or NFκB may play a decisive role. Both ERK and NFκB have been implicated in the development of radioresistance following radiotherapy (17,18). ERK1 is activated following clinically used dose of ionising radiation and enhances the proliferation of normal human diploid cells (19). Likewise, NFκB is known to be inhibited by indomethacin, a well-known radiosensitizer (20). Persistent activation of these molecules would protect the cell from apoptosis and if it harbours a mutation or chromosomal aberration, will lead to carcinogenesis. Whether this pattern is unique to $^{16}O^{7+}$ ion as has been observed here or is common to all heavy ions has to be seen.

REFRENCES

(A) P. Varadkar, M. Krishna and N.C. Verma, *Antioxidants and Redox Signaling* **3**, 483-492 (2001).

(B) Y.R. Chen *et al*, *J. Biol. Chem.* **271**, 31929-31936 (1996).

(C) R.R.Weichselbaum *et al*, *Int. J. Radiat. Oncol. Biol. Phy*. **30**, 229-234 (1994).

(D) I. Szumiel,*Radiat. Res.* **150,** S92-S101 (1998).

(E) C. Lucke-Huhle, E.A. Blakely, P. Chang and C.A. Tobias, *Radiat. Res.* **79**, 97-112 (1979).

(F) G.E. Woloschak and C.M. Chang-Liu, *Radiat. Res.* **124,** 183-187 (1990).

(G) B. Marples and K.A. Skov, *Radiat. Res.* **146,** 382-387 (1996).

(H) M.A. Ritter, J.E. Cleaner and C.A. Tobias, *Nature* **266,** 653-655 (1977).

(I) A.V. Prasad, N. Mohan, B. Chandrasekar and M.L. Meltz, *Radiat. Res.* **138,** 367-372 (1994).

(J) P.K. Schmitt-Ullrich *et al*, *Radiat Res.* **153,** 245-257 (2000).

(K) A.M. Rauth, *Basic Science of Oncology* (I.F. Tannock and R.P. Hill, Eds.), pp.106-124. Pergamon Press, Elmsford, NY, 1987.

(L) C. Wildmann *et al*, *Mol. Cell. Biol.* **18,** 2416-2429 (1998).

(M) P.L. Jones, D. Ping and J.M. Boss, *Mol. Cell. Biol.* **17,** 6970-6981 (1997).

(N) C-Y. Wang, M.M. Mayo and A.S. Baldwin Jr., *Science.* **274,** 784-787 (1996).

(O) J.C. Ghosh *et al*, *Radiat. Res.* **153,** 305-311 (2000).

(P) M.A. Stevenson *et al*, *Cancer Res.* **54,** 12-15 (1994).

(Q) A. Yacoub *et al*,. *Int. J. Radiat. Biol*. **77,** 1067-1078 (2001).

(R) C-Y. Wang, M.W. Mayo and A.S. Baldwin Jr., *Science,* **274,** 784-787 (1996).

(S) S. Carter *et al*, *Oncogen,* **16,** 2787-2796 (1998).

(T) C.M. Bradbury *et al*, *Cancer Res.,* **61,** 7689-7696 (2001).