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Role of NADPH Oxidase in Ionizing Radiation-Induced Nuclear Damage

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1. Introduction

While it is well known that ionizing radiation (IR) can damage nuclei, its underlying mechanism has been poorly characterized. Given that IR can elevate cellular levels of reactive oxygen species (ROS) and also that ROS can induce DNA damage, ROS may be involved in the IR-induced nuclear damage. However, this possibility has not been directly addressed.

The mechanism whereby IR induces ROS generation is unclear. In the case of other types of stimuli, such as growth factors, cytokines, and chemokines, NADPH oxidase, a plasma membrane enzyme that can generate superoxide dismutase, was shown to be stimulated [1]. Therefore, this study investigated the possible role of NADPH oxidase in IR-induced nuclear damage.

2. Materials and Methods

2.1 Cell Culture, DNA Transfection, and Treatments

Human Jurkat leukemia cells were cultured in RPMI 1640 media supplemented with 10% heat-inactivated fetal bovine serum and getamicin (50 μg/ml). Cells were resuspended at a concentration of 3 x 10^5 cells/ml, and irradiated with indicated doses of γ-rays. Where specified, LY294002 (LY), catalase, or diphenyleneiodonium (DPI) were added at the indicated concentrations. For DNA transfection, the dominant negative mutant of p85 subunit of phosphoinositide 3-kinase (PI3K) (PI3K-M) was cloned into the pTRE vector, and was delivered into Jurkat cells by electroporation using the Tet-Off gene expression system (Clontech, Palo Alto, CA). Alternatively, siRNA of Nox, a catalytic subunit of NADPH oxidase, was cloned into pSUPER vector, and delivered into the cells by the same method. The transfected cells were selected by using 1 mg/ml G418 sulfate. The PI3K-M transfectants were maintained in the presence of 2 μg/ml doxycycline to minimize basal expression of the cloned PI3K-M. The transfectants were washed, cultured in doxycycline-free media for 2 days to induce the PI3K-M expression, and then received the indicated treatments.

2.2 Analysis of Cellular ROS Levels

Treated and untreated control cells were exposed to 50 μM 2',7'-dichlorofluorescein diacetate (DCFH-DA, Molecular Probes, Eugene, OR) for 5 min at 37^0C. Cell-associated DCF fluorescence levels were analyzed by flow cytometry (FL-1 channel) [2].

2.3 PI3K Assay

Cells were lysed in Tris-HCl (20 mM, pH 8.0), 137 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1% NP-40, 10% glycerol, 2 mM sodium orthovanadate, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1mM phenylmethylsulfonyl fluoride. The lysates were clarified, and equal amounts of the lysate proteins (400 μg) were immunoprecipitated with an antibody against the p85 subunit of PI3K (Upstate Biotechnology, Lake Placid, NY). The immune complexes were washed twice with 1% NP-40, 1 mM sodium orthovanadate, and PBS (pH 7.4); twice with 100 mM Tris-HCl (pH 7.5), 500 mM LiCl, and 1 mM sodium orthovanadate; and twice with 150 mM NaCl and 50 mM Tris-HCl (pH 7.2). The kinase reactions were initiated by adding 5 mg/ml of L-α-phosphatidylinositol (Sigma) in 20 mM HEPES (pH 7.4), 5 mM $MnCl_2$, 10 μM ATP, 10 μCi γ-[^{32}P]ATP, and 2.5 mM EGTA. After 20 min incubation, the reactions were quenched by adding 1 M HCl. The phospholipids were extracted using a 1 : 1 mixture of chloroform and methanol, and separated by thin-layer chromatography [3].

2.4 Micronucleus Assay

Cells were treated with cytochalasin-B for 24 hr, washed in 0.075M KCl, and resuspended in a mixture of methanol and glacial acetic acid. The fixed cells were air-dried, and stained with 10% Giemsa. The micronuclei were scored in 300 binucleated cells using a 1000X magnification.

3. Results

3.1 IR Elevates Cellular ROS Levels

Jurkat cells were irradiated with 2.5 Gy of γ-rays, and cellular ROS levels were compared by DCF fluorescence. The obtained fluorescence were: untreated cells, 4.9; treated cells, 9.5. This suggests that the IR treatment induce ROS generation in Jurkat cells.

3.2 PI3K is not Involved in the ROS Induction.

In the case of growth factor-induced signaling, PI3K was shown to mediate ROS induction. Under the present experimental conditions, IR induced PI3K activation.

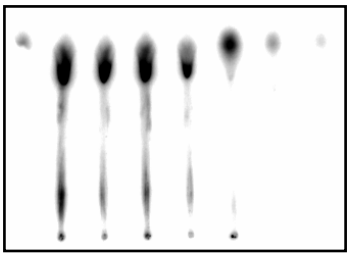

Figure 1. PI3K Activation. PI3K activity was analyzed from 0, 2.5 min, 5 min, 15 min, 30 min, 1 hr, 4 hr, 18 hr (left to right) after irradiation.

However, the addition of LY or the expression of PI3K-M did not significantly reduce IR-induced DCF fluorescence. Control, 10.4; LY, 12.1; PI3K-M, 11.7. This suggests that PI3K does not mediate IR-induced ROS generation.

3.3 NADPH Oxidase Mediates IR-induced ROS Generation.

In contrast, the expression of Nox1 siRNA significantly reduced IR-induced ROS accumulation.

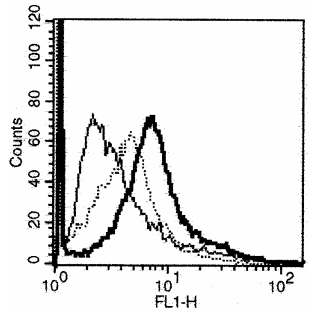

Figure 2. Effect of Nox1 siRNA on ROS production. Left, control; Middle, Nox1 siRNA plus irradiation; Right, irradiation only.

This suggests that NADPH oxidase is involved in IR-induced ROS generation.

3.4 NADPH Oxidase and ROS Mediates IR-induced Nucleus Damage.

Finally, the treated and untreated control cells were analyzed for micronucleus formation.

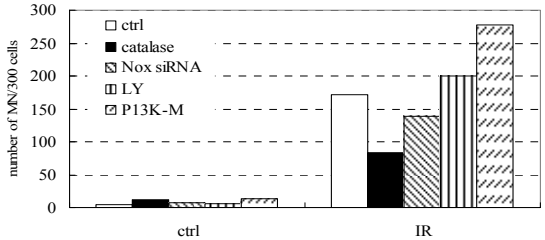

Figure 3. Micronucleus assay. Control and indicated transfectants were irradiated with 2.5 Gy of γ-rays for 24 hr in the presence or absence of catalse or LY. Micronucleus assay was performed.

The data shows that catalse and Nox1 siRNA, but LY and PI3K-M not, prevent IR-induced micronucleus formation.

4. Conclusion

This study shows that NADPH oxidase mediates the ability of IR to damage nucleus by generating ROS. Although IR activates PI3K, this enzyme does not appear to be involved in ROS generation under the present experimental conditions.

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