Detection of the Level of Reactive Oxygen Species Induced by Ionizing Radiation in Cells

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1. Introduction

Ionizing radiation is closely related to human health and has a wide variety of practical applications in modern life of human beings. It can give rise to direct and/or indirect effects in biological objects such as cells, tissues, organs and individuals. By definition, the direct effect is referred to interaction between photon and DNA molecule, whereas the indirect effect is mediated by the reactive oxygen species (ROS) generated by radiolysis and subsequent reaction. It has been reported that ROS produced after exposure to IR can react with cellular materials such as DNA, proteins, carbohydrates and lipids [1].

ROS is free radicals such as the superoxide anion, hydroxyl radicals and the non-radical hydrogen peroxide. Cells generate ROS during aerobic metabolism. Excessive production of ROS can lead to oxidative stress, genetic alteration and even cell death. It has been reported that ROS plays a critical role in radiation-induced cell injury. Thus, it is of great interest to determine the radiation-induced ROS level. Many kinds of methods to detect the level of ROS have been developed so far. They include the chemiluminescence of liminonol and lucigenin, cytochrome c reduction, ferrous oxidation of xylenol ogrange and 2'-7'-dichlorogdihydrofluorescein diacetate (DCFH-DA) [2]. Among others, the DCFH-DA assay is one of the most widely used methods for measuring the formation of intracellular ROS.

The aim of the present study is to evaluate the effective protocol for measuring ROS in the cells exposed to gamma-rays by means of the DCFH-DA assay.

2. Materials and Methods

Human hepatoma cells were used in the experiment. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Isla, NY, USA) with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT, USA), 100 U/mL penicillin and 100 U/mL streptomycin (Gibco, Grand Islan, NY, USA). The cells were incubated under the normal condition at 37^{o}C with 5% CO_2.

After incubation, the cells were irradiated with 0, 1, 5 and 10 Gy of gamma-rays from a ^{60}CO source (7.4 PBq of capacity; Advanced Radiation Technology Institute of Korea Atomic Energy Research Institute).

Cell survival after irradiation was analyzed by the clonogenic assay. After irradiation, cells were incubated for 1, 2, and 3 days, respectively. Then, the cells were trypsinized, counted, and seeded in triplicated 100-mm dishes (100 and 500 cells per dish) and incubated for 14 days to allow for colony growth. After 14 days, colonies were fixed with 70% ethanol, stained with crystal violet (0.3%) and counted using a counter. The survivorship (%) was calculated as (number of colonies/number of cells plated)/(number of colonies for corresponding sham-irradiated control/number of cells plated) x 100.

For the comet assay, the cells irradiated with gamma-rays were immediately isolated with trypsinization and prepared for the alkaline comet assay previously described [3]. The comet assay was carried out under dark and cool conditions.

For evaluation of the DCFH-DA assay, the cells were plated in 96-well plates at $1x10^4$ cells per well. The cells were incubated for 1 day and treated with DCFH-DA solution before and after irradiation. Each well was added 10 μM of DCFH-DA solution, kept for 30 min under dark condition and washed with PBS. One hour after incubation, the fluorescence intensities of the wells were measured by the fluorescence micro plate reader.

3. Results and Discussions

Cell viability was measured by the trypan blue assay to determine gamma radiation-induced cytotoxicity in the cells. The cells were incubated for 1 day, irradiated with 0 and 5 Gy. As reported elsewhere, irradiation decreased the percentage of cell viability after irradiation with 5 Gy, confirming that gamma radiation is cytotoxic to the cells.

For quantification of radiation-induced genotoxicity in the cells, DNA damage was measured by the comet assay. The cells were incubated for 1 day, irradiated with 0 and 5 Gy, and immediately isolated to avoid DNA repair. As shown in Fig. 1, the fragmented DNA formed a typical comet tail-like pattern after irradiation with 5 Gy. This result indicated that gamma-rays induced DNA damage in the cells.

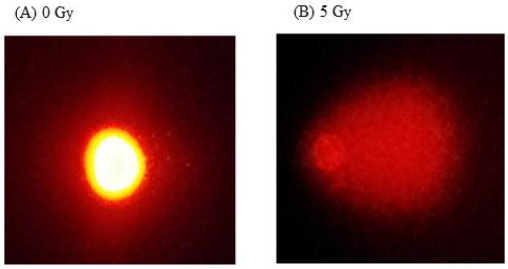

Fig. 1. DNA damage measured by the Comet assay.

For measurement of ROS level induced by ionizing radiation in the cells, comparison experiments were done on the cells treated with DCFH-DA solution before and after irradiation. There were random changes of fluorescence intensity in the treatment after irradiation. This result meant that this protocol was not appropriate for determination of radiation-induced ROS. On the other hand, the fluorescence intensity was increased in a dose-dependent manner when the cells were treated with the DCFH-DA solution before irradiation.

Conclusions can be drawn from the experimental results of this study. In order to properly measure the ROS level in the cells exposed to ionizing radiation, the cells should be treated with the DCFH-DA solution before irradiation.

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