BL-27B/2012G746

Bystander Effects in HUVEC Cells Exposed to X-ray Microbeams

Hiroshi Maezawa*[1], Noriko Usami[1], Yoshihiro Uto[2] and Katsumi Kobayashi[1]
[1]Institute of Materials Structure Science, KEK, Tsukuba 305-0801, Japan
[2]University of Tokushima Graduate School, Tokushima 770-8506, Japan

1 Introduction

Bystander effect is a third type of radiation action in addition to classification of direct- and indirect-action. Several biological effects such as lethality, mutagenesis, apoptosis and chromosomal aberration *in vitro* were investigated with high-LET radiation microbeams. Microbeams can irradiate selectively nucleus and cytoplasm of cells. Bystander effects induced by X-rays may be different from that by high- LET radiations.

The irradiation system of cells using monochromatic X-ray microbeams was developed at PF, KEK (Kobayashi *et al*., 2001, Usami *et al*., 2006). Several investigators have reported the presence of bystander effects in neighboring cells after irradiation of target cells with X-ray microbeams. It was shown that migration of glioma cells might be enhanced by bystander effects after microbeam irradiation of a small number of cells [3].

The aim of the present study was to investigate induction of lethality and migration on unirradiated human umbilical vein endothelial (HUVEC) cells by bystander effects via cells exposed to X-ray microbeams.

2 Experiment

HUVEC cells (passage 2~4, Cancer Research Institute of Kanazawa University) were exponentially grown in EGM-2 (LONZA) supplemented 10% fetal bovine serum, hydrocortisone, hFGF-B, VEGF, IGF-1, ascorbic acid, hEGF, GA-1000 and heparin. Cells were plated on the polypropylene membrane of the stainless steel dish and cultured overnight at 37ºC. Cell nuclei stained with Hoechest33258 (1 μM) were targeted with 5.35 keV microbeam collimated with 10μm x 10μm at BL-27B. Dose (Gy) was calculated using exposure (C/kg) and conversion factor (34.1 Gy/(C/kg)). After irradiation cells were incubated in fresh medium for 24 h and then assayed for the colony formation to assess cell survival. Cells were treated with 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO, nitric oxide (NO) scavenger), dimethyl sulfoxide (DMSO, hydrogen peroxide radical scavenger), and L-N^6-(1-iminoethyl) lysine hydrochloride (L-nil, induced nitric oxide synthase inhibitor) for 24 h after irradiation. Cell migration was assessed with the transwell assay method. Under side of membrane (8 μm pore) of transwell was coated with fibronectin. Cells were plated on the upper side of membrane, cultured in 24-well dish and then fixed with ethanol. After stain with crystal violet the number of cells moved to under side of membrane were counted under the optical microscope.

3 Results and Discussion

The decided numbers (10 and 100) of the nuclei, which were randomly selected on the dish, were exposed to microbeams at 0.44, 0.88 and 4.4 Gy. Significant reduction of survival in bystander cells was observed in the case of irradiation of 100 nuclei at 4.4 Gy (Fig.1). If carboxy-PTIO and DMSO were present in the medium after irradiation, survival of HUVEC cells did not reduce as compared with sham control. In addition L-nil protected lethal effects by cells irradiated with microbeams.

Migration of untargeted-cells after irradiation of 100 cells at 4.4 Gy was not significantly different from the control.

The present results suggested that both NO and OH radical in HUVEC cells contribute to bystander lethal effect induced by irradiation with X-ray microbeams. Migration of untargeted cells might be influenced by the kind of cell strains and the molecules induced in cells irradiated with X-rays.

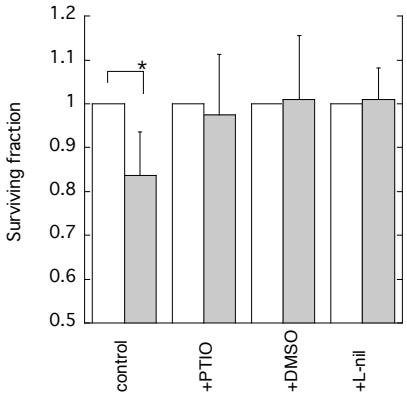

Fig.1. Survival of bystander HUVEC cells after irradiation of 100 cells/nuclei at 4.4 Gy with X-ray microbeams. After irradiation cells on dish were treated with carboxy-PTIO, DMSO and L-nil for 24 h at 37ºC.
* p<0.01, statistical analysis was performed with student's t test, .

References

[1] K.Kobayashi *et al*., Nucl. Instrum. Methods A467-8, 1329 (2001).
[2] N.Usami *et al*., Radiat. Prot. Dosimetry, 122, 307 (2006)
[3] H.Maezawa *et al*., Photon Factory Activity Report 2011 #29, Part B, 341 (2012)

*mae_525rb@yahoo.co.jp