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Proteomics Study on the Variation of Protein Expression and Phosphorylations caused by Radiation in Cultured Grial Cells

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Abstract We have reported the radiation adaptive response in cultured glial cells conditioned with a low dose and subsequently challenged with a high dose of X-rays. The radiation adaptive response was evident for glial cells cultured from young rats, while not from aged rats. In order to investigate the mechanisms of the decline of adaptive response with aging, we examined the protein profiles of variously aged rat astrocytes in response to low dose irradiation. After 0.1 Gy-irradiation, the relative intensity of protein spot changed on two-dimensional (2-D) gels compared with control gels. As a result, we detected the protein spot, which increased temporarily 3 hrs after low dose irradiation.

Introduction

Low dose radiation has been reported to induce hormesis [1, 2] and radiation adaptive response [3]. Radiation adaptive response is a biological defensive response that is induced by single low dose irradiation under certain conditions, and has been observed widely from prokaryotes to eukaryotes. However, the molecular mechanisms of adaptive response remain obscure.

We have reported the radiation adaptive response in cultured glial cells and the effects of aging [4, 5]. We studied the molecular mechanism of the adaptive response, and clarified the participation of protein kinase C, ataxia-telangiectasia mutated (ATM), and DNA-dependent protein kinase (DNAPK) in the adaptive response. However, the mechanism of the decrease of adaptive response with aging remains unclear. Therefore, we try to reveal the mechanisms of the decline of adaptive response with aging.

Genomic and transcriptomic profiles have been determined for a large number of potential biomarkers [6], but the functional components of a biological system are proteins and not genes. Therefore focusing on proteins has certain advantages compared to focusing on mRNA [7, 8]. Thus, we examined protein profiles of glial cells in response to low dose irradiation and analyzed the expression and phosphorylation of cellular proteins of glial cells.

Materials and Methods

Cell culture

Wistar rats (females; 1, 4, 9, and 24 months old) were obtained from the Laboratory Animal Facilities of the Tokyo Metropolitan Institute of Gerontology. The Animal Care and Use Committee of the Tokyo Metropolitan Institute of Gerontology approved all experimental procedures involving animals in this study. Primary culture of glial cells taken from the rat hippocampus was carried out according to a previously described conventional procedure [4]. Cells were cultured in MEM supplemented with 10% fetal bovine serum and penicillin-streptomycin at 37 °C under a humidified atmosphere containing 5% CO_2/95% air. At confluence, the cells were trypsinized, collected, resuspended, and plated in 10 cm dishes and used for the experiments. The cell cultures were >95 % immunopositive against glial fibrillary acidic protein (GFAP), a marker for astrocytes.

Radiation adaptive response

X-ray irradiation of cells was performed with a Hitachi X-ray irradiator. Cells were divided to four groups as follows; 0 Gy alone, a low dose of 0.1 Gy alone, a high dose of 2 Gy alone and a low dose followed by a high dose. The controlled cells (0 Gy alone) were treated with sham irradiation, which were taken out of a CO_2 incubator and left under air at room temperature during irradiation. Cells were incubated for 2 days after the additional irradiation and were counted with a Coulter counter after trypsinization. The growth inhibition caused by additional irradiation was measured and the effects of low dose pre-irradiation on the growth inhibition were used as an indicator of radiation adaptive response.

Proteomics

Protein extraction and 2-DE were performed as previously reported (see also the URL, http://proteome.tmig.or.jp/2D/2DE_method.html), with slight modifications [9-11]. Briefly, the harvested cell suspension was supplemented with three volumes of the extraction buffer. After disruption of cells by sonication, the cell extract was loaded on to reswollen gel strips with IPG (pH 4-7) and IEF was performed. Next, each equilibrated strip was mounted on a polyacrylamide gel slab (7.5% T, 3% C) and SDS-PAGE was performed. The protein spots were stained with silver, SYPRO Ruby, or ProQ Diamond, a specific stain of phosphoproteins, and analyzed by using PDQuest 2-D image processing

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software.

Results and Discussion

The growth of astrocytes was inhibited by 2 Gy-irradiation in both groups with and without pre-irradiation. However, in astrocytes from young rats, the pre-irradiation of cells suppressed the growth inhibition due to 2 Gy, indicating that pre-irradiation of caused radiation adaptive response. However, pre-irradiation did not significantly influence growth inhibition in cells from aged rats, suggesting that the radiation adaptive response was not observed in aged rat cells [4]. Furthermore, when the intervals between pre- and additional irradiation was 3 hrs, the radiation adaptive response was observed, while it was not observed in the intervals of 24 hrs even in cells from young rats.

From proteomics study, we detected the spot, which increased 3 hr after 0.1 Gy-irradiation and recovered 24 hr after irradiation. The temporary increase of this spot due to 0.1 Gy-irradiation was observed in cells from young rats, while not in cells from aged rats. From analysis of phosphoprotein profiles, low dose radiation phosphorylated β-actin, and de-phosphorylated elongation factor I-β and β-actin. The biological meanings of the phosphorylation and de-phosphorylation of these molecules due to low dose irradiation must be clarified from now on.

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