Immunohistochemical Study of Flotillin-1 in the Hippocampal Neurons of Irradiated Mice

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Abstract

We analyzed the expression of flotillin-1 in the hippocampus of mice, using a ^{60}Co γ-ray source for irradiation (single dose of 15 Gy), to investigate cellular responses.

Immunohistochemistry showed that flotillin-1 was constitutively expressed in some CA1 pyramidal cells and dentate gyrus (DG) granular cells in the hippocampus of normal mice. Expression in pyramidal cells at 24 h after irradiation of the CA1 was increased significantly, but no significant difference was observed in the intensity of the immunoreactivity in the granular cell layer of the DG between control and irradiated mice. The results suggest that irradiation affects expression of flotillin-1 in hippocampus CA1 pyramidal cells, and that flotillin-1 might be involved in resistance to irradiation.

Key words : Brain; Flotillin-1; Hippocampus Irradiation

Introduction

Flotillin-1 and the associated protein flotillin-2 (epidermal surface antigen) are important structural proteins in lipid rafts (Bickel *et al.*, 1997). These proteins have been added to an expanding list of proteins that are co-localized at the caveolae, including protein kinase C alpha, Ras, Rap, Src-like kinase, and glycosyl phosphatidyl-inositol-linked receptors (Harris *et al.*, 2002; Razani *et al.*, 2002).

Flotillins are particularly enriched in the detergent-insoluble, glycolipid-enriched membrane domains that are involved in both signal transduction and vesicular trafficking (Hooper, 1999; Lang *et al.*, 1998), as well as in protein tyrosine nitration in the brain (Dremina *et al.*, 2005). In the diseased brain, flotillins are associated with the production of beta-amyloid in flotillin-enriched lipid rafts, which may reflect the progression of Alzheimer's disease (Kokubo *et al.*, 2000; Lee *et al.*, 1998). Flotillin-1 is localized predominantly to catecholaminergic nerves in the substantia nigra in a rat model of Parkinson's disease (Jacobowitz & Kallarakal, 2004). Furthermore, flotillin-1 is known to be involved in neuroprotection against oxidative stress (Wakasugi *et al.*, 2004), and in the course of experimental autoimmune encephalomyelitis (Kim *et al.*, 2006).

Cranial radiation therapy is very important for the successful treatment of several cancer-originating central nervous system (CNS) diseases (Monje & Palmer, 2003). Although

radiation is used for CNS cancer treatment, side effects, such as glial cell activation, neuronal cell apoptosis, disruption of the blood brain barrier, and white matter necrosis, can occur (Kyrkanides *et al.*, 1999 Monje & Palmer, 2003). In the mature brain, apoptotic cell death by radiation is observed mainly in the hippocampus, especially the dentate gyrus (DG) granular cells (Monje & Palmer, 2003). However, little is known about the cellular response to irradiation of the hippocampus, particularly changes in lipid raft proteins such as flotillin-1. The aim of this study was to localize flotillin-1 in the hippocampus of mice after irradiation.

Materials and methods

Animals

C57BL/6 mice were obtained from Orient (Gyunggi, Korea) and bred in our animal facility. Male mice (7-8 weeks old) were used in this study. All experiments followed accepted ethical guidelines.

Irradiation

Irradiation was carried out using a ^{60}Co γ-ray source (10,000 Ci; ^{60}Co Irradiation Facility, Applied Radiological Science Research Institute, Cheju National University, Korea). The whole body of the mouse was irradiated with a single dose of 8 Gy. After irradiation, the animals were taken back to the animal facility and cared for in a routine manner for 24 h.

Tissue sampling

The rats were euthanized 24 h postirradiation (PI) (n = 5). Control animals were not irradiated (n = 5). Brain samples were processed for paraffin embedding after fixation in 4% paraformaldehyde in phosphate-buffered saline (PBS; pH 7.4).

Immunohistochemistry

Paraffin-embedded sections (5 μm) were deparaffinized, treated with citrate buffer (0.01 M, pH 6.0) in a microwave for 15 min, and then treated with 0.3% hydrogen peroxide in methyl alcohol for 20 min to block endogenous peroxidase activity After three washes with PBS, the sections were incubated with 10% normal goat serum and then with primary reagents, including polyclonal anti-flotillin-1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-glial fibrillary acidic protein (GFAP) (Dako, Glostrup, Denmark) for 1 h at room temperature. Immunoreactivity was visualized using the avidinbiotin peroxidase complex (Vector Elite; Vector Laboratories, Burlingame, CA, USA). The peroxidase reaction was developed using a diaminobenzidine substrate kit (Vector Laboratories).

Results

Radiation increases GFAP immunoreactivity in mouse hippocampus

We examined the effect of ionizing radiation on the intensity of GFAP immunoreactivity, which is widely used as a marker of astrocyte activation or reactive gliosis after irradiation (Hwang *et al.*, 2006). Low intensity GFAP immunoreactivity was constitutively detected in astrocytes in the hippocampus of normal mice (Fig. 1A, B). In

irradiated mouse hippocampus, intense immunostaining occurred for GFAP in astrocytes (Fig. 2C, D), which related to the irradiation injury described previously (Hwang *et al.*, 2006).

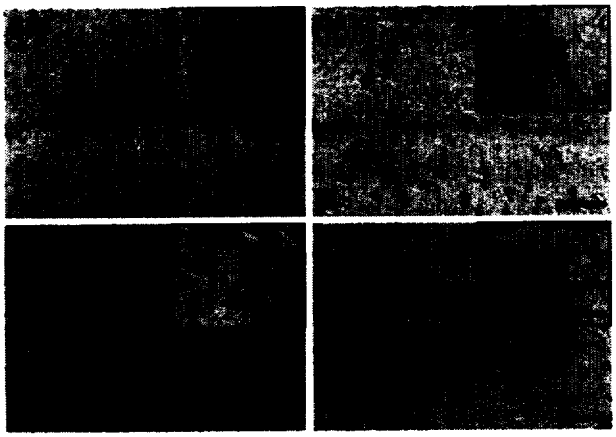

Fig. 1. Immunohistochemical staining of GFAP in the hippocampus CA1 (A, B) and DG (C, D) of control (A, C) and irradiated mice at 24 h postirradiation (B, D). GFAP was weakly detected in some astrocytes in the hippocampus CA1 (A) and DG (C) of control spinal cords, while GFAP immunoreactivity was intense in astrocytes in the hippocampus CA1 (B) and DG (D) of irradiated spinal cords. Inserts show a higher magnification of the areas indicated by the arrows. Counterstained with hematoxylin. Scale bars: main pictures, 100 ㎛ insets, 20 ㎛.

Localization of flotillin-1 in the hippocampus of irradiated mice

Flotillin-1 immunoreactivity was detected constitutively in CA1 pyramidal cell layers of the hippocampus in the control mice (Fig. 2A, C). Moreover, the immunoreactivity was constitutively observed in some granular cells of the DG in the controls. After irradiation, expression of flotillin-1 was elevated in the CA1 of the hippocampus at 24 h, while no significant change in expression was observed in the granular cell layer of the DG. The intensity of the flotillin-1 immunoreactivity in the normal control and irradiated mice is summarized in Table 1.

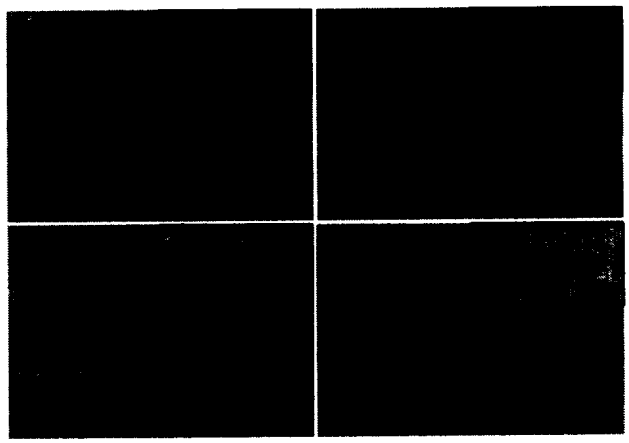

Fig. 2. Immunohistochemical staining of flotillin-1 in the hippocampus CA1 (A, B) and DG (C, D) of control (A, C) and irradiated mice at 24 h postirradiation (B, D). Flotillin-1 was weakly detected in some neurons in the hippocampus CA1 (A, arrowhead) of control spinal cords, while flotillin-1 immunoreactivity was intense in the neurons in the hippocampus CA1 (B, arrowhead) of irradiated spinal cords. Positive immunoreactivity for flotillin-1 was observed in the cytoplasm of neurons (A, B, arrowheads). No significant difference was detected in the intensity of the immunoreactivity in the DG neurons between the control (C) and irradiated mice (D). Inserts show a higher magnification of the areas indicated by the arrows. Counterstained with hematoxylin. Scale bars: main pictures, 100 ㎛ insets, 20 ㎛.

Table 1. Immunoreactivity of flotillin-1 in each region of the hippocampus of normal and irradiated mice

Regions	Normal mice	Irradiated mice
CA1 pyramidal cell layer[a]	+[b]	+++
DG granular cell layer	+	+

[a]Three different sections from three animals in each group were examined by two blinded observers.

[b]The presence of immunoreactive cells in the neuronal cells of each region is expressed as

negative (-), <10 cells positive (+), 10-30 cells (++), and >30 cells (+++) at 20 magnification.

Discussion

This is the first study to demonstrate that irradiation increases the expression of flotillin-1 in mice, mainly in the neurons of the hippocampus pyramidal cell layer. This finding implies that increased expression of flotillin-1 plays an important role in cellular responses to irradiation in CNS tissues (especially the hippocampus). Although the biological relevance of flotillin-1 expression in CA1 pyramidal cells of the hippocampus is not clear, the general consensus is that flotillin-1 recruits neuroglobin to lipid rafts as a means of preventing neuronal death (Wakasugi *et al.*, 2004).

Neuroglobin is widely expressed in the cerebral cortex, hippocampus, thalamus, hypothalamus, and cerebellum (Wakasugi *et al.*, 2004). The DG, which receives neocortical input via the entorhinal cortex, is a remarkably dynamic structure and a major site of postnatal/adult neurogenesis (Monje & Palmer, 2003). The hippocampal granular cells are so sensitive to radiation that a single low dose to the brain of a mature animal is enough to stop hippocampal neurogenesis (Monje & Palmer, 2003). However, postmitotic neurons are considered more resistant to ionizing radiation (Monje & Palmer, 2003; Peissner *et al.*, 1999). Thus, it is highly possible that the increase in expression of flotillin-1 in CA1 pyramidal cells is associated with the recruitment of neuroglobin to the hippocampus of irradiated rats, and that it has a selective neuroprotective role in resistance to irradiation.

This study revealed that flotillin-1 is significantly enhanced in the hippocampus pyramidal cell layer after irradiation, suggesting that flotillin-1 is involved in resistance to irradiation.

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