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Expression of *Jun*B Induced by X-rays in Mice

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Objective To explore *Jun*B gene expression in spleen cells of mice after the whole body irradiation as well as in normal hematopoietic and leukemia cells in the primary culture after different dosages of X-ray irradiation. **Methods** Spleen cells were isolated from the mice irradiated with 3 Gy X-rays. Primary cultured cells from mice were incubated in different intervals after X-irradiation at different dosages. Total RNA was extracted from the cells and the fluctuation of *Jun*B mRNA level was assessed by the RNA ratio of *Jun*B/β-actin measured by quantitative Northern blot hybridization. **Results** After the mice were exposed to 3 Gy X-rays irradiation, *Jun*B expression in spleen cells was remarkably and rapidly increased, and reached its peak 0.5 h later in C3H/He mice and 1 h later in Balb/c mice. In the primary culture of normal spleen and leukemia cells, *Jun*B mRNA levels increased 30 min after irradiation. The enhanced levels of *Jun*B mRNA were returned to a normal level within 240 min after irradiation. **Conclusions** *Jun*B gene is responsive to ionizing irradiation and is induced at immediate-early phase after the stimulation. This suggests that the *Jun*B gene plays an important role in the early process of the cells against radiation.

Key words: *Jun*B; Whole body irradiation; Northern blot hybridization

INTRODUCTION

Ionizing radiation stimulates various kinds of biological reactions in mammalian cells. When cells were irradiated, numerous mammalian genes were induced as radiation-response genes[1,2]. It has been shown that the mechanism of radiation-response genes involved in the recovery from radiation-damage in mammalian cells is not yet completely clear. However studies have shown that several cytokines can facilitate the recovery from such damage via activation of signal transduction systems.

We previously found that mRNA for cytokine IL-1β was expressed transiently within 30 min after irradiation in the primary culture of spleen cells *in vitro*[3]. The splenic IL-1β mRNA level was increased from several hours to several days after the mice received whole-body irradiation *in vivo*, and the peak varied in a dose-dependent manner[4]. These findings implied that the time courses were different between the *in vitro* and the *in vivo* experiments because of the signal transduction mechanism primed by the expression of distinct radiation-responsive genes. Vincenti *et al.* found that the elevated IL-1β mRNA expression was closely related to *Jun*B expression[5]. To analyze the unique mechanism

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against ionizing radiation, kinetic studies on the expression of *Jun*B gene in spleen cells *in vivo* and *in vitro* were performed.

MATERIALS AND METHODS

Animals and Irradiation

Specific pathogen-free male C3H/He and Balb/c mice at 8-10 weeks old inbred in National Institute Radiological Sciences, Japan, were used in the study. X-ray was generated at 200 kVp/20 mA and filtered through 0.5 mm-thick Cu and Al plates. The exposure rate was monitored with a Victreen R meter. Dose rates of 0.682 and 0.899 Gy/min were used for whole-body irradiation and cultured cells, respectively.

Whole Body Irradiation in Mice

After the whole-body exposure to sub-lethal dose of 3 Gy X-rays of both C3H/He and Balb/c inbred mice, the spleens were removed 0.5, 1, 2 or 4 h after the irradiation and frozen at -80℃. Total RNA was extracted by the guanidium/hot-phenol method.

Preparation of Normal Cultured Spleen Cells and Cell Irradiation

Normal spleen cells from 5 mice were pooled with MEM α medium (Sigma) containing 5% fetal calf serum (FCS). After the removal of red-blood cells by NH_4Cl-treatment and washing 3 times with the same medium, 1.5 mL of the suspension containing 2×10^5 cells was plated in 35 mm plastic Petri dishes and pre-incubated at 37℃ in 5% CO_2 for 3 h. The cells were irradiated at room temperature with 0, 0.2, 0.5, 1.0, 2.0, or 4.0 Gy X-rays and incubated at 37℃ in 5% CO_2 for 30 min, 60 min, 120 min or 240 min. Immediately after incubation, the cells were chilled at 4℃, pelleted, and stored at -80℃. For positive control, addition of hydrogen peroxide (H_2O_2) at a final concentration of 100 g/mL into the culture was replaced by irradiation.

Preparation of Leukemia Cells and Irradiation

Cell line L8704 was adhesive macrophage-like leukemia, established from C3H/He mice with radiation-induced leukemia[6]. The cells were cultured with RPMI1640 medium (Gibco) containing 10% FCS. Irradiation to the cells was carried out with 0, 0.2, or 2.0 Gy of X-ray. The further incubation and storage conditions were the same as described above.

Preparation of RNA and Quantitative Northern Blot Hybridization

RNA was extracted from cells using the guanidium/hot-phenol method[7], subjected to electrophoresis in 1% agarose gel after glyoxylation, and electro-transferred on a positively charged nylon membrane (IBI, Optiblot)[8]. The membrane was pre-hybridized with 50% formamide (Bethesda Research Laboratories) containing 1% sodium dodecyl sulfate, 0.1% Ficoll 400 (Pharmacia), 0.1% bovine serum albumin (Sigma, fraction V), 0.1% polyvinyl pyrrolidone, 100 μg/mL sonicated salmon sperm DNA, 50 mmol/L Tris-HCl (pH 7.5) and 50 mmol/L sodium phosphate/5×SSC at 42℃ for 1 h. Hybridization was performed by the incubation of the blot membrane with radio-labelled DNA probes for 16 h. To prepare the DNA probes, a template DNA for protooncogene *Jun*B obtained from Japanese Cancer Research Resources Bank in size of 1.5 kb and a template DNA for human β-actin gene

purchased from Wako Pure Chemical Industries (Osaka, Japan) were used. Radioactive DNA probes were prepared by the randon-primer DNA labelling kit (Bethesda Research Laboratories) with template DNAs and ^{32}P-α-dCTP and diluted to a specific activity of about 10^8 cpm/μg DNA. After hybridization, the membrane was washed twice at 60℃ with 2×SSC containing 1% sodium dodecyl sulfate for 30 min and exposed to the imaging plate (Fuji Photo Film Co.). Quantitative auto-radiograms were obtained by 2-dimensional measurement of the imaging plate with the BAS2000 system (Fuji Photo Film Co.). Each sample was analyzed for mRNA expressions of both *Jun*B and β-actin on the same gels, and the ratio between them was calculated to represent the mRNA expression level of *Jun*B. All experiments were repeated twice.

RESULTS

Splenic JunB Expressed Immediately After Whole-body-irradiation in Mice

Levels of *Jun*B mRNA in spleen from both C3H/He and Balb/c inbred mice exposed to sublethal dose of 3 Gy X-rays were measured by quantitative Northern blot hybridization. As shown in Fig. 1, the immediate-early expression of *Jun*B mRNA was remarkable, and reached its peak within 0.5 h in C3H/He mice and 1 h in Balb/c mice after the irradiation. Meanwhile, the increase of expression was not observed in another member for fos/*jun* family protooncogene *Jun*D. In contrast, c-*jun* mRNA was not detected among all the samples (data not shown).

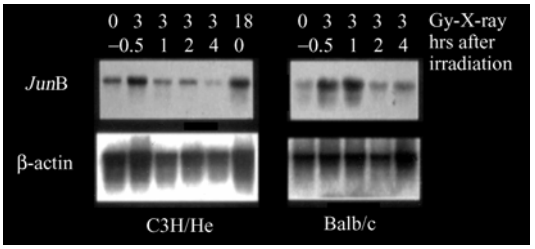

FIG. 1. *Jun*B gene expression in both C3H/He and Balb/c mice shown by Northern blot hybridization after sub-lethal dosage of 3 Gy X-rays irradiation. *Jun*B mRNA increased rapidly and remarkably, reached its peak within 0.5 h in C3H/He mice, 1 h in Balb/c mice.

JunB Gene Expressed in Normal Cultured Spleen Cells After Different Dose X-ray Irradiation

The auto-radiograms of Northern blot hybridization were quantitatively analyzed. Relative level of *Jun*B mRNA was determined by calculating the RNA ratio of *Jun*B/β-actin. The fluctuation of *Jun*B mRNA level following the radiation was revealed by comparing with the ratio per *Jun*B level in un-irradiated cells (Fig. 2). In sham-irradiated group, increase in the level of *Jun*B mRNA was not observed. When H_2O_2 was added, *Jun*B gene expression was induced with 30 min and returned to the normal level 60 min after the addition. Similar immediate-early induction of *Jun*B gene was observed in the irradiation group. Level of *Jun*B mRNA reached its peak within 30 min by 0.2, 2.0, and 4.0 Gy of X-ray, or within 60 min by 0.5 and 1.0 Gy irradiation. All of them decreased gradually and returned to the normal level within 240 min.

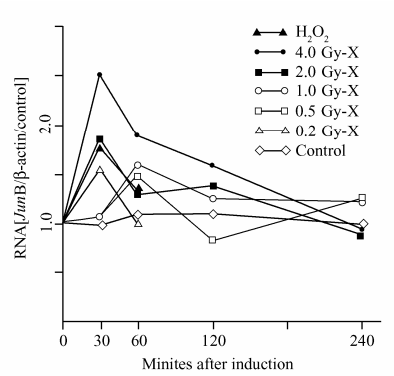

FIG. 2. *Jun*B gene expression in normal cultured spleen cells shown by quantitative analysis of Northern blot after different dosage of X-rays irradiation. *Jun*B mRNA was induced immediately, reached its peak within 30 to 60 min, then gradually returned to normal level within 240 min.

*Jun*B *Gene Expressed in Leukemia Cells After Different Dose X-ray Irradiation*

The same experiment was done as described above on leukemia cells. In sham-irradiated group, *Jun*B gene expression was not induced at any time. In the H_2O_2 group, *Jun*B gene expression was induced within 30 min, and returned to the normal level within 60 min. In the irradiation group, both 0.2 and 2.0 Gy X-rays irradiation induced *Jun*B expression, the level of *Jun*B mRNA peaked within 30 min and dropped to the same level of the sham-irradiation group within 60 min (Fig. 3).

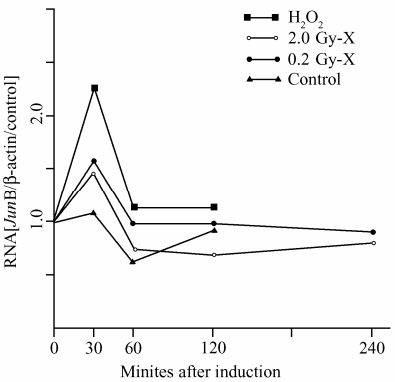

FIG.3. *Jun*B gene expression in leukemia cells shown by quantitative analysis of Northern blot after different dosage of X-rays irradiation. *Jun*B mRNA was induced immediately, peaked within 30 min, and then dropped to the same level of sham-irradiation group within 60 min.

DISCUSSION

*Jun*B, a member of fos/jun family protooncogene, is a major component of the leucine zipper transcription factor of the activated protein-1 (AP-1), and a main target of mitogen-activated transduction pathway[9,10]. It was found that *Jun*B was rapidly induced by a wide variety of extracellular stimuli such as serum, growth factors, phorbol esters (TPA) and

activators of protein kinase A (PKA)[11]. However, induction mechanism by ionizing radiation is not known. In the present study, induction of the *Jun*B expression was shown by quantitative Northern blot analysis. The results showed that the level of *Jun*B mRNA in spleen was rapidly elevated by whole-body irradiation of 3 Gy X-rays. It reached the peak within 30 min in C3H/He mice and 1 h in Balb/c mice (Fig. 1). Similarly, the immediate-early induction of *Jun*B gene was observed in both primary cultured spleen cells (Fig. 2) and L8704 leukemia cells (Fig. 3) after X-ray irradiation at different doses. These indicated that at least in mice, *Jun*B was a responsive gene against ionizing radiation, since radiation at a very low dose could induce significant *Jun*B expression.

It has been reported that the inverted repeat sequence positioned at -57 to -50 from the transcriptinal initiation site of *Jun*B gene can mediate the induction of *Jun*B promoter by TPA and cAMP[12]. Signal transduction via the adenylate cyclase/cAMP system involves activation of cAMP-dependent protein kinase A (PKA). Activated PKA transduction message to the cell nucleus as protein kinase C (PKC) does react to TPA or receptor-linked phospholipid turnover. Activation of these kinases could finally result in the modulation of the activity of transcription factors such as NF-kappaB or members of the AP-1 gene family[13]. Ionizing radiation, as extracellular stimuli, could induce numerous cytokines including IL-1 that has a protective function against radiation[14], suggesting the emergence of the protection mechanism against radiation damage. Studies on the regulatory mechanism of IL-1β and *Jun*B genes are important to explore and control the endogenous mechanism for radiation protection. In our previous studies, IL-1β mRNA in spleen cells was obviously induced after 3 Gy X-rays whole body irradiation, reaching its peak in 5 to 7 d[4]. When spleen cell suspension was irradiated *in vitro*, IL-1β mRNA transient accumulation was observed within 30 min. The difference in time course implies that the mechanism of whole body irradiation is complicated. In the present study, *Jun*B mRNA increased immediately after irradiation, and reached its peak within 30 min, indicating that *Jun*B gene plays an important role in the process of message transduction, particular in whole body irradiation.

Recently, it has revealed that the *Jun*B protein replaced by c-Jun protein of AP-1 could result in decrease of transcriptional activity[15]. If the immediate-early induction of *Jun*B gene resulted in enhancement of the protein, endogenous AP-1-like transcription factor would be trans-repressed, leading to the decreased or delayed induction of cytokine genes including IL-1 gene. This presumption seems to be conflicting with our previous finding that IL-1 gene was induced immediately after irradiation. Following explanation is possible to clarify such conflict. Firstly, knowledge of the activity of AP-1 families is limited, since the fos/*jun* family is consisted of various proteins. Message of c-*jun* was not detected in mouse hematopoietic cells in the study, suggesting that there is a very low level of full-active AP-1 protein in the cells. If the cells are regulated by heterologous AP-1-like protein containing different members of *jun* family protein, it is not clear whether the *Jun*B protein represses the AP-1-like transactivator. Secondly, induction rate of IL-1 gene is varied by the cell-type and conditions, suggesting that there are multiple regulation mechanisms for IL-1 gene expression. Recently, we have isolated the far-upstream region sequence of IL-1 gene that might contribute to the immediate-early induction[16]. Since the sequence excludes known regulative motify such as AP-1 target, it is suggested that the IL-1 gene is regulated not only by the previously known mechanism including AP-1 and NF-kappaB, but also by other activating proteins. At present, it is still unclear why the expression of a gene for a cytokine as well as radio-protector should have an immediate responsive phase after X-irradiation, although most of the genes are induced immediately by X-ray encoding nuclear factors. It is necessary to further study the sensitive sites of *Jun*B gene.

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