

Tumor suppressor p53 response is blunted by low-dose-rate radiation

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Abstract

To estimate the effects of space radiation on health of space crews, we aimed to clarify whether γ -ray-irradiation at a low-dose-rate interferes in a p53 centered signal transduction pathway induced by radiation in human cultured cells and CB-17 Icr^{+/+} mice. *In vitro* experiments, the human cultured squamous cell carcinoma cells (SAS/*neo*) were examined for cellular levels of p53 and Bax, and the incidence of apoptosis after irradiation at a low-dose-rate (1 mGy/min) or a high-dose-rate (1 Gy/min). It was found that challenging irradiation-induced apoptosis was depressed by chronic irradiation at 1.5 Gy for 25 h with the depression of p53 and Bax accumulation. *In vivo* experiments, a significant suppression of Bax and apoptosis induced by challenging irradiation at 3.0 Gy was observed when the mice were pre-irradiated chronically at 1.5 Gy for 25 h in the spleen of CB-17 Icr^{+/+} mice. These findings suggest that chronic pre-irradiation suppressed p53 function through radiation-induced signaling and/or p53 stability.

KEYWORDS: p53, Bax, apoptosis, radioadaptation.

1. Introduction

In space, there are two main factors of interest, that is, space radiation and microgravity. Space radiation has characteristics of low-dose-rate. When space crews are exposed to space radiation during long stay in space, it is possible that they receive some risks from space radiation. However, there are no significant data to assess the effects of space radiation on health of space crews. On the other hand, p53 is well known to play important roles in cancer suppression through induction of cell growth arrest, DNA repair or cell death in response to radiation [1]. We have already reported that p53 accumulation was induced in several organs of whole body-irradiated mice and rats receiving low-dose radiation less than 0.5 Gy in ground experiment [2, 3]. Further, chronic irradiation at a low-dose-rate induces the accumulation of p53 and WAF1 in a different manner from that induced by high-dose irradiation in ground experiment [4]. In the present study, we analyzed the effect of pre-irradiation at a low-dose-rate on p53 response or apoptosis incidence after challenging high-dose irradiation. Furthermore, we examined the effect of the pre-irradiation at a low-dose-rate on the accumulation of Bax and apoptosis in spleen of whole-body irradiated mice.

2. Materials and Methods

Western blot analysis of p53 and Bax were applied in cultured human squamous cell carcinoma cells (SAS/*neo* cells having the wild-type p53 function) 6h after chronic γ -irradiation (1 mGy/min) or acute X-irradiation (1 Gy/min). Induction of apoptosis was analyzed by detection of apoptotic bodies with Hoechst33342

staining 48h after irradiation. Specific pathogen free 5-week-old female mice of CB-17 Icr^{+/+} strains were irradiated with X-rays at 3.0 Gy (1 Gy/min for 3 min) immediately after conditioning chronic irradiation with γ -rays at 1.5 Gy (1 mGy/min for 25 h). The samples were fixed for immunohistochemistry 12h after irradiation. Bax on formalin-fixed paraffin-embedded sections were stained by the avidin-biotin peroxidase complex method using HISTOFINE SAB-PO(R) kit (Nichirei Co., Tokyo, Japan). Apoptotic cells in the sections were detected by staining with ApopTag *in situ* detection kit[®] (Intergen Co., NY).

3. Results

Effect of chronic pre-irradiation on radiation-induced apoptosis in human cultured cells

Although accumulation of p53 and Bax was not observed by chronic irradiation at 1.5 Gy for 25 h, the levels of p53 and Bax increased up to 6 h after irradiation with X-rays at 5.0 Gy in SAS/*neo* cells. In contrast, challenging acute irradiation at 5.0 Gy immediately after chronic irradiation at 1.5 Gy for 25 h did not apparently induce an accumulation of p53 and Bax. Challenging acute irradiation at 5.0 Gy for 5 min induced efficiently apoptotic cells (about 10%) stained with Hoechst33342 (Fig. 1Ac), though chronic irradiation at 1.5 Gy for 25h slightly induced apoptosis (about 1%) (Fig. 1Ab). However, chronic pre-irradiation depressed the apoptosis one fourth as compared with the case of challenging acute irradiation alone (Fig. 1Ad).

Effect of chronic pre-irradiation on radiation-induced Bax and apoptosis in the spleen of mice

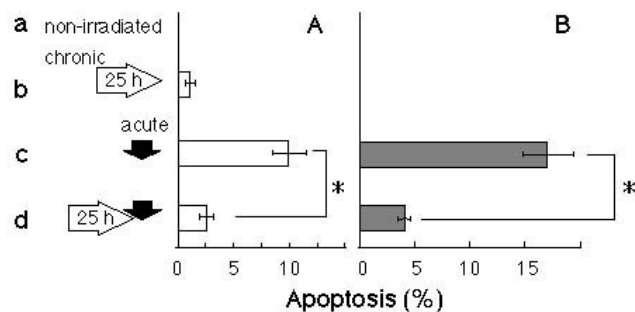


Fig. 1 – Effect of pre-irradiation with low-dose-rate on radiation-induced apoptosis in human cultured SAS/*neo* cells (A) and CB-17 Icr^{+/+} mouse spleen (B). ⇨; chronic irradiation at (1 mGy/min for 25 h); ↓, (1 Gy/min for 3 min (A) or 5 min (B)). Error bars indicate standard deviations. *, indicates a highly significant difference ($P < 0.01$) by Student's *t*-test.

Pre-irradiation itself did not induced Bax- and apoptosis-positive cells in the spleen (Fig. 1Bb). The accumulation of Bax and the incidence of apoptosis were detected in the spleen of CB-17 Icr^{+/+} after X-ray irradiation at 5 Gy (Fig. 1Bc). On the other hand, chronic pre-irradiation at 1.5 Gy for 25h depressed the induction of Bax accumulation and apoptosis induced by challenging acute irradiation in the spleen of mice (Fig. 1Bd).

4. Discussion

In the present study, we found that chronic pre-irradiation with γ -rays at a low-dose-rate can attenuate the induction of Bax and apoptosis induced by challenging acute-irradiation in human cultured cells and mice spleen. In the present stage, we consider two possible mechanisms for the non-responsiveness of p53 after chronic pre-irradiation at a low-dose-rate as follows. The first mechanism is the lack of emergency signals, which is supported by the findings that adaptive response is related to damage reduction by the induction of radical detoxification and/or repair system [5]. The second one is the perturbation of signaling, which is supported by the fact that low-dose radiation activates protein kinase C (PKC), shortly after exposure to ionizing radiation [6]. PKC is reported to up-regulate the transcriptional activity of p53 for *Bax* gene expression [7]. Thus, suppression of PKC activity by chronic pre-irradiation may cause down regulation of *Bax* gene expression. Although these possible mechanisms are proposed, it remains unfortunately unclear what

induces the non-responsiveness of cells to acute challenging irradiation after chronic irradiation. Since chronic pre-irradiation interfered with the p53 response, it is possible that the frequencies of mutation and chromosomal aberration may be changed under different conditions of irradiation. If p53-dependent radiation-induced apoptosis can prevent carcinogenesis, chronic pre-irradiation at a low-dose-rate might increase the frequency of cancer events. We expect that the present findings will provide useful information for the care of crews' health.

5. Conclusions

Chronic conditioning irradiation with low-dose-rate suppressed Bax-mediated apoptosis. These findings obtained from human cultured cells and mice spleen suggest that elucidation of the effects of chronic irradiation at a low-dose-rate is a highly important issue in research concerning radiation-induced signal transduction.

Acknowledgements

This study was funded in part by "Ground Research for Space Utilization" promoted by NASDA and Japan Space Forum.

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