J. RADIAT. RES., 43: SUPPL., S261–S264 (2002)

Hypergravity Modifies the Signal Transduction of Ionizing Radiation through p53

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Hypergravity / Ionizing radiation / p53 / Signal transduction

To determine the possible effect of hypergravity to modify the signal transduction of ionizing radiation, we analyzed the accumulation of p53 and the expression of p53-dependent genes, Waf-1 and Bax, using the western blot analysis. Hypergravity (20 × g) induced the accumulation of p53 in the human glioblastoma cell line A172 after 3 h of incubation. Low-dose (0.5 Gy) irradiation to the cells accumulated p53 1.5 h after irradiation, and induced Waf-1 and Bax. Under the condition of hypergravity (20 × g), the peak of p53 accumulation was shifted from 1.5 h to 3 h after irradiation, and the inductions of Waf-1 and Bax were suppressed entirely. These results indicate that hypergravity modifies the signal transduction of ionizing radiation through p53 in the cells.

INTRODUCTION

Microgravity causes considerable changes in the expression of various genes, such as c-fos, c-jun[1)], the cytokines IL-1 and IL-2[2)], and the tumor suppressor gene p53[3)]. Ikenaga et al.[4)] reported that the frequency of sex-linked recessive lethal mutations in *Drosophila melanogaster* was significantly higher in male flies sent into space on the US Space Shuttle *Endeavor* than in control flies kept on earth. From this result, they suggested that the effects of space radiation are greatly enhanced under microgravity.

The space environment is characterized by microgravity and space radiation. However, it is difficult to reproduce the microgravity conditions and space radiation on Earth. The alternative is to use hypergravity generated by a centrifuge, and also ionizing radiation generated by an X-ray machine. Cogoli et al.[5)] reported that hypergravity stimulated the proliferation of human lymphocytes. Hypergravity also enhanced the expression of *c-myc*[6)], *c-fos* and *erg-1* as well as the activity of protein kinase C[7)] in human and mouse cells. These genes and protein are concerned with cell growth and differentiation. We wondered if p53 and the p53-dependent genes may affected by hypergravity. The protein of p53 controls cell growth as well as cell death. As a transcription factor, p53 causes a halt in cell-cycle progression by the upregulation of certain gene products, including Waf-1, which has been identified as a potent inhibitor of several cyclin-Cdk complexes[8)]. p53 is also able to induce cell death by apoptosis, and the Bax gene, which is transcriptionally activated by p53, may play an important role in this response[9)]. The signal transduction of p53 is very sensitive to not only various forms of radiation, such as X-ray or heavy-ions[10)], but also various stresses, such as hypoxia, heat shock, low pH and osmotic shock[11)]. We thus examined p53 signal transduction to study the influence of hypergravity on the effect of X-ray radiation in a human glioblastoma cell line.

MATERIALS AND METHODS

Cell Culture

Human glioblastoma A172 cells (provided by JCRB, Tokyo, Japan), bearing the wild-type p53 gene, were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum.

Centrifugal Hypergravity

Cells in tissue culture flasks (25 cm^2, Iwaki Glass, Tokyo,

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Japan) were centrifuged in a swing-type centrifuge designed for cultured cells (Kawasaki Heavy Industries, Inc., Kobe, Japan) in an incubator at 37°C.

Irradiation

Cells in the growth phase were irradiated with a 200-kV X-ray source (Toshiba X-ray machine, Tokyo, Japan) at a dose rate of 0.308 Gy/min, at room temperature.

Western Blot Analysis

The cells were washed once with PBS, and the total protein was extracted. Thirty milligrams of protein were subjected to a Western blotting analysis. After electrophoresis on a 10% (for p53) or 15% (for p21 and Bax) polyacrylamide gel containing 0.1% sodium dodecyl sulfate, the proteins were electrophoretically transferred to a nitrocellulose membrane. The proteins were then incubated for 1 h with specific monoclonal antibodies against human p53 (Anti-Human p53 Oncoprotein, Upstate Biotechnology, Inc., Lake Placid, NY), human p21 (Purified mouse anti-human Sdi1 monoclonal antibody, Pharmingen, San Diego, CA), and human Bax (N21, Santa Cruz Biotechnology Inc., CA). Other details of the Western blotting procedure have been described previously[12]. The protein level was analyzed using an Amersham-enhanced chemiluminescence system (ECL). The band densities were measured using a Macintosh computer with the IPLab Spectrum and IPLab Gel programs (Scanalytics, Inc., Vienna, VA).

The experiments were performed at least three times, and showed the same tendency. Typical data are shown in each Figure.

RESULTS AND DISCUSSION

We examined whether hypergravity may induce the accumulation of p53. Cells of A172 were centrifuged at various gravities (10, 20, 30 and 40 × g) and incubated at 37°C. Hypergravity at 20 × g induced p53 accumulation after 3 h of incubation. Other hypergravity less effectively induced p53 accumulation (Fig. 1). We then examined the effect of hypergravity at 20 × g on the signal transduction of p53 by ionizing radiation. First, we observed the accumulation of p53 in A172 cells in response to a low dose (0.5 Gy) of radiation. Fig. 2 shows the results of western blotting for p53 and the relative amount of protein by measuring of the band densities. The p53 protein was found to have accumulated in the cells 1.5 h after irradiation. Next, after irradiation, the cells were incubated under hypergravity of 20 × *g*. The peak of p53 accumulation was observed to shift from 1.5 h to 3 h (Fig. 2), indicating that hypergravity had modified the p53 signal pathway for ionizing radiation.

We then examined Waf-1 and Bax, which are located downstream from p53. Although Waf-1 was induced by a low dose of ionizing radiation, the induction was suppressed entirely by incubation under hypergravity of 20 × *g* (Fig. 3). Similarly, Bax was induced by a low dose of ionizing radi-

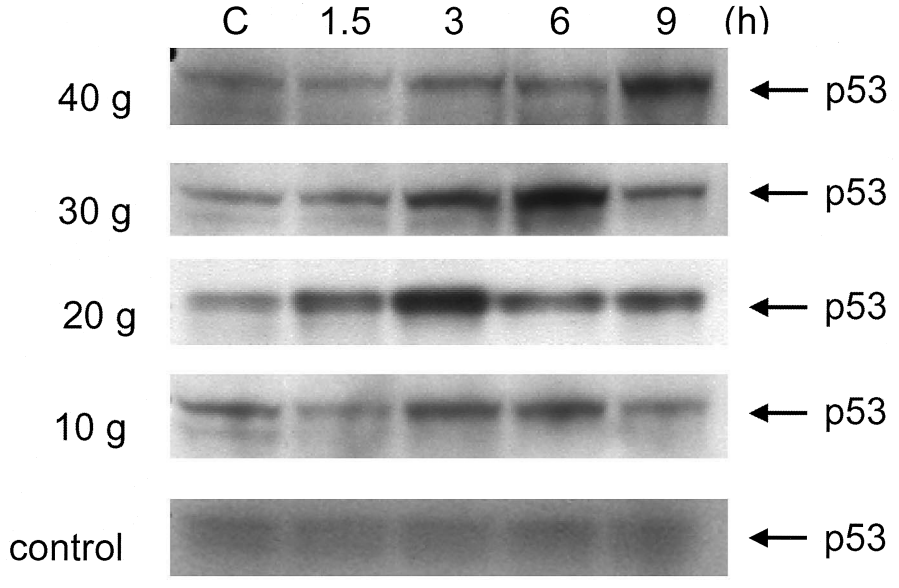

Fig. 1. Accumulation of p53 by hypergravity.
A172 cells were centrifuged by various gravity (10, 20, 30 and 40 × g) and incubated at 37°C. The accumulation of p53 was detected by Western blot analysis.

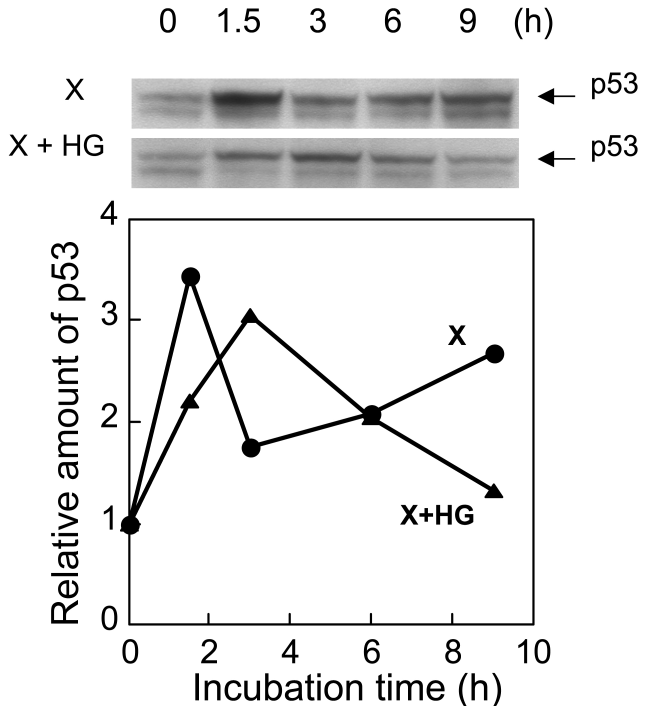

Fig. 2. Western blot analysis of p53.
A172 cells were exposed to 0.5 Gy of X-ray radiation and incubated under ground conditions (X) or 20 × *g* of hypergravity (X+HG).

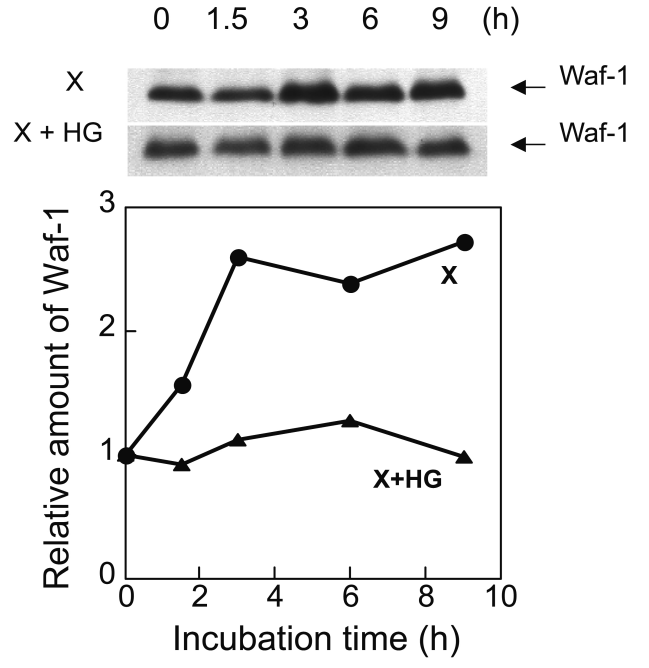

Fig. 3. Western blot analysis of Waf-1.
A172 cells were exposed to 0.5 Gy of X-ray radiation and incubated under ground conditions (X) or 20 × *g* of hypergravity (X+HG).

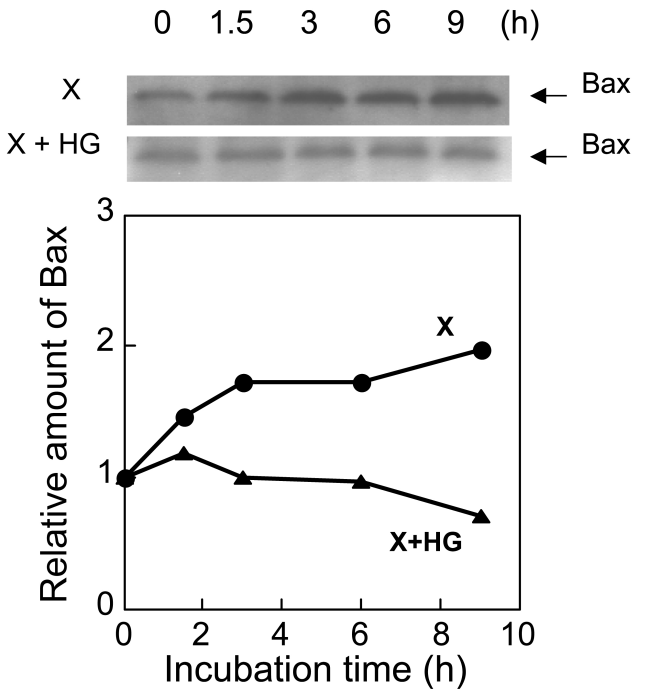

Fig. 4. Western blot analysis of Bax.
A172 cells were exposed to 0.5 Gy of X-ray radiation and incubated under ground conditions (X) or 20 × *g* of hypergravity (X+HG).

ation, and the induction was also entirely suppressed by hypergravity (Fig. 4).

These data indicate that hypergravity suppresses the signal transduction of p53 induced by ionizing radiation. Suppression of the p53 function may modify the effect of irradiation in these cells. Hypergravity at 20 × g, itself, induced the accumulation of p53 after 3 h (Fig. 1), but suppressed the accumulation of p53 after 1.5 h by irradiation. This indicates that the signal pathway of p53 accumulation by hypergravity and irradiation may be different. Modifications of p53, such as phosphorylation at ser-20, play a role in p53 stabilization by irradiation[13]. Hypergravity may alter such a modification to stabilize p53 by radiation. Other modifications of p53, such as phosphorylation at ser-15, activate p53 and induce many genes[14]. Hypergravity does not induce Waf-1 or Bax effectively by itself (data not shown), and may actually suppress such a modification to activate p53 by radiation. Takahashi[15] reported that pre-irradiation at a low dose-rate suppressed the signal transduction of p53. Hypergravity may also affect the p53 response as pre-irradiation at a low dose-rate. We considered that the signal transduction of p53 induced by ionizing radiation and hypergravity must crosstalk with each other[16]. Because p53 is a guardian of the genome[17] and has a role in DNA repair, hypergravity may increase the mutation rate induced by

radiation. There is some evidence that a space flight causes genetic changes in living organisms, including humans[18–20]. A space flight is characterized not only by the presence of cosmic radiation and microgravity, but also by the occurrence of hypergravity during a transient flight with a space shuttle. The present work concerning suppression of the p53 signal pathway by hypergravity indicates that it may enhance genetic changes in astronauts during space flight.

ACKNOWLEDGEMENTS

This work was funded in part by the "Ground Research for Space Utilization" program promoted by NASDA and the Japan Space Forum, and "Grants-in Aid for Scientific Research (NO. 14380255)" program promoted by the Ministry of Education, Science, Sports and Cultures, Japan.

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Received on May 8, 2002
Revision on September 9, 2002
Accepted on November 1, 2002