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THE INDUCTION OF A TUMOR SUPPRESSOR GENE (*p53*) EXPRESSION BY LOW-DOSE RADIATION AND ITS BIOLOGICAL MEANING

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ABSTRACT-- I report the induced accumulation of wild-type p53 protein of a tumor suppressor gene within 12 h in various organs of rats exposed to X-ray irradiation at low doses (10- 50 cGy). The levels of p53 in some organs of irradiated rats were increased about 2- to 3-fold in comparison with the basal p53 levels in non-irradiated rats. Differences in the levels of p53 induction after low-dose X-ray irradiation were observed among the small intestine, bone marrow, brain, liver, adrenal gland, spleen, hypophysis and skin. In contrast, there was no obvious accumulation of p53 protein in the testis and ovary. Thus, the induction of cellular p53 accumulation by low-dose X-ray irradiation in rats seems to be organ-specific. I consider that cell type, and interactions with other signal transduction pathways of the hormone system, immune system and nervous system may contribute to the variable induction of p53 by low-dose X-ray irradiation. I discussed the induction of p53 by radiation and its biological meaning from a aspect of the defense system for radiation induced cancer.

Extrapolation is generally employed to estimate the risks of low-dose radiation, as no direct data on such risks in the form of health indicators or cytogenetic indices are available[1]. Studies on tumor incidences in mice[2] and epidemiological data on cancer incidence in populations exposed to low radiation doses in areas with high back-ground radiation[3,4] have provided good support for the threshold model of such risks. However, the use of health indicators as well as cytogenetic indices appears to be unhelpful for gaining an understanding of the mechanisms underlying the threshold for cancer incidence[5]. Therefore, it is deemed necessary to search for evidence of cellular responses to low-dose radiation at the molecular level. We have focused on the response of a tumor suppressor gene product, p53 protein, since recent studies have revealed that this may play an important role in carcinogenesis[6] and radiosensitivity[7]. Data obtained using cultured cell systems have demonstrated that marked accumulation of p53 protein, found to associate to various kinds of proteins and bind to a specific DNA sequences located at upstream of p53 targeted genes (Fig. 1). In addition, the depression of several genes is dependent on p53 through binding to TATA box binding proteins. p53 is also a consequence of a post-transcriptional event[8]. The accumulation of cellular content of p53 is induced by various types of stress, such as heat[9] and DNA-damaging agents, including ionizing radiation, UV light[10] through the activation of protein kinase C. Therefore, these chain-chemical reactions after stress is called as p53-dependent signal transduction pathways. Elevated levels of p53 protein induce G1 arrest[11,12] or apoptotic cell death in some cell types[13] (Fig.1). p53 is also directly and indirectly involved in both DNA repair machinery and DNA replication through association with transcription factors such as ERCC3[14], and replication protein A[15], or by inducing GADD45[16] (Fig. 1).

Although these are many reports on cultured cells irradiated with X-rays at high doses, there are no data on the *in vivo* p53 response in various organs of animals after whole-body exposure to low dose radiation.

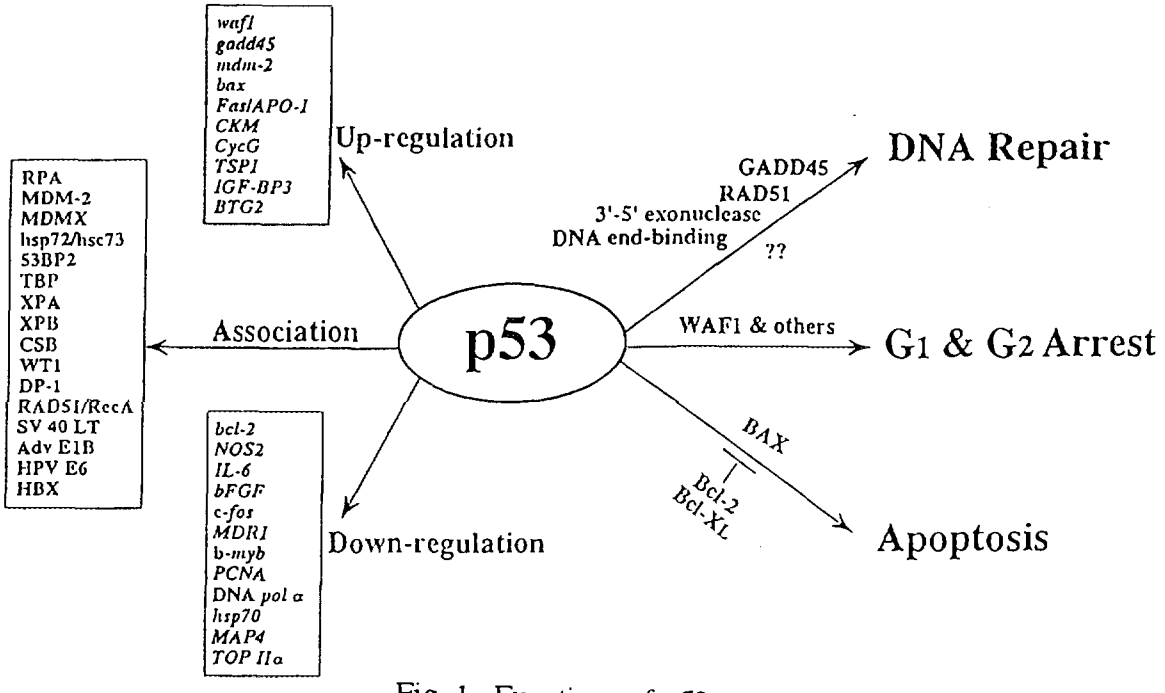

Fig. 1. Functions of p53.

MATERIALS AND METHODS

Animals and X-ray irradiation. Six-week-old F344 rats were used, and divided randomly into irradiation or non-irradiation (control) groups. Three rats per time-point were used. They were irradiated with X-ray at a dose rate of 50 cGy/min (Radiation Biology Center of Kyoto University). After receiving a total dose of 10-50 cGy, they were kept at 25°C with a 12-h light/dark cycle and allowed free access to food and tap water.

Preparation of protein samples from rat organs. The rats were killed by cervical dislocation at various times (-36 h) after irradiation, and their organs were removed, frozen immediately and stored at -80°C until analysis. The tissues were then pulverized by freeze-fracturing and suspended in RIPA buffer containing SDS. The bone marrow was washed out from the femurs with RIPA buffer and also subjected to freeze-thawing. The insoluble cell debris was removed, the supernatants were collected and the protein concentration was quantified using protein assay reagent and a spectrophotometer.

Western blotting analysis with anti-p53 monodonal antibody and quantitation of p53 protein. The total protein samples (20 μg) were subjected to SDS-polyacrylamide gel electrophoresis, then transferred electrophore-tically to Immo-bilon™ PVDF membrane. The membranes were incubated with an anti-p53 monoclonal antibody, PAb 421, then treated with a horseradish peroxidase-conjugated anti-mouse IgG antibody. The sensitivity of the visualization of the p53 bands was enhanced using the BLAST blotting amplification system. The densities of the p53 bands were measured using a personal image analyzer. The amount of p53 protein was derived by taking the average value of the three rats in each group.

RESULTS AND DISCUSSION

A group of kinetic diagrams of p53 accumulation in all the organs of the exposed rats examined are shown in Fig. 2. The time course of p53 accumulation can be classified into four kinetic patterns. We defined the pattern for the small intestine as pattern 1, the response of which to low-dose X-ray irradiation was rapid with p53 accumulation evident 3 h after

exposure to all three doses. The elevated p53 level was maintained for 24 h, then increased further after 36 h. The characteristics of pattern 2 were an increase in the level of p53 protein 6 h after irradiation with all three doses, maintenance of this elevated level by 24 h, then the start of a drop or maintenance of the same level for up to 36 h. The bone marrow, liver, adrenal gland and brain fell into this category. Three organs, the spleen, hypophysis and skin, were classified into the category of pattern 3, in which a significant p53 response was observed only after one specific dose, rather than all three doses. The doses which resulted in significant p53 accumulation in the rat skin, hypophysis and spleen were 10, 25 and 50 cGy, respectively. No p53 accumulation in the organs showing pattern 3 was observed after the other two doses. Pattern 4 was defined as no p53 response after any of these three doses as shown in the testis and ovary. In this study there were striking differences in the p53 responses among the organs. This organ-specificity may be attributable to differences in cell differentiation, differences in the mechanisms of response in cells of various organs to X-rays or differing interactions with other signal transduction pathways of the hormonal and immune system. Involvement of the nervous system in the interaction with the p53 signal transduction pathway cannot be excluded, as apparent fluctuations in the in-creased level of p53 were observed in the rat brain, which is one of the most sensitive organs to low-dose X-rays as demonstrated by stress-induced changes. With regard to organs that respond to only one specific dose, the mechanism underlying this phenomenon is still unclear. The apparent randomness of the effect may be due to complex interactions among the nervous, immune and/or hormonal systems of the body.

Exposure of mammalian cells to ionizing radiation induces transcription of a variety of proteins involved in DNA repair, cell cycle arrest and cell death[17-20]. Marked increases in the levels of p53 protein have been observed in mammalian cultured cells exposed to γ-rays, UV[10,21]. Here we have obtained firm evidence of increases in cellular p53 level in rat organs in response to low-dose X-rays irradiation. We assume that even the low doses of X-rays caused DNA damage. Since an activation of p53 dependent apoptosis was induced in certain cultured cells by high-dose ionizing irradiation[10]. Also low-dose X-ray-induced apoptosis in the rat cerebellum has been demonstrated in several studies. This induction of apoptosis by low-dose X-ray irradiation may be due to p53 accumulation as shown in Fig. 2.

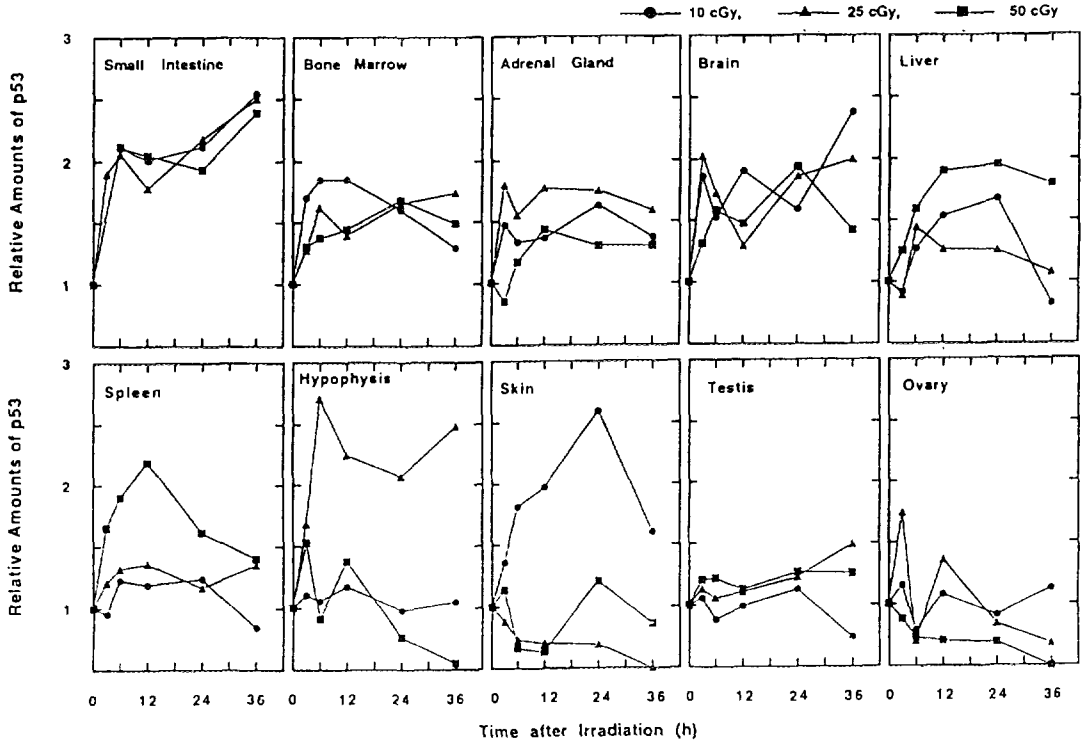

Fig. 2. Kinetics of p53 accumulation in the organs of rats exposed to low-dose X-rays. The p53 protein levels are expressed as ratios to that in non-irradiated rats. The amount of p53 at each time point was derived by taking the average of the values in three rats.

Further *in vivo* studies of DNA repair in animals exposed to low-dose irradiation are also needed to obtain a full understanding of the biological significance of the p53 accumulation, as p53 appears to have multifunctional role in cell cycle control, DNA repair and DNA replication through association with transcription factors such as ERCC3 and replication protein A, or by inducing the expression of GADD45. Therefore the induction of p53 by low dose radiation may contribute to the prevention of cancer event, because there are threshold in several kinds of radiation-induced cancer.

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