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RADIATION-MEDIATED INDUCTION OF P53 PATHWAY IN THE RAT HIPPOCAMPUS

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Abstract

Ionizing radiation (IR) is commonly used in the treatment of brain tumors but it can cause significant damage to surrounding normal brain tissue. In this study, heads of young Wistar rats (18 days old) were subjected to a single dose of gamma irradiation (10 Gy) similar to that used in prophylactic brain irradiation of children with acute lymphoblastic leukemia. The kinetics of apoptosis associated proteins (p53 and Bax) were examined by Western blotting and RT-PCR. We observed that p53 mRNA expression was unchanged after irradiation, while induction of p53 protein was rapid, leading to the accumulation of p53 protein in the cytoplasm. In addition, Bax mRNA and protein levels were also increased following cranial irradiation. These results indicate that cranial irradiation, used in terms of prophylactic therapy, is associated with activation of the p53 system, alongside with induction of positive apoptosis regulator Bax.

Introduction

Under normal conditions the tumor suppressor protein p53 is a short-lived protein that is maintained at low level in the cell. In many tissues, irradiation results in the up-regulation of p53 protein by a post-translational stabilization mechanism, presumably not followed with change in the mRNA level [1]. Active p53 protein induces the transcription of several downstream genes that, in turn, can trigger a variety of biological processes such as cell cycle arrest, apoptosis and DNA repair. The overall consensus is made that Bcl-2 family members are crucial in p53-mediated apoptosis. This family includes two categories of proteins: those functioning as suppressors of apoptosis (Bcl-2, Bcl-x_L, Bcl-w) and those that promote apoptosis (Bax, Bcl-x_S, Bak, Bad, Bik and Noxa). Pro-apoptotic members like *bax*, *bak* and *noxa* are transcriptionally upregulated by p53, while expression of anti-apoptotic *bcl-2* is suppressed by p53 [2]. The relative ratios of these various pro- and anti-apoptotic members of the Bcl-2 family have been shown to determine the ultimate sensitivity or resistance of cells in response to ionizing irradiation.

The aim of the present study was to investigate the expression of p53, in the hippocampus of head-irradiated rats, both at the level of mRNA and protein. Simultaneously, expression of positive apoptosis regulator Bax, as downstream p53-target gene, was explored in the same animal model of CNS prophylactic brain irradiation.

Experimental

The experiments were performed using previously established animal model for CNS prophylactic therapy of childhood acute lymphoblastic leukemia (ALL) [3]. Animals at the age of 18 days were divided into two groups: sham-irradiated controls (C) and irradiated animals (IR). IR animals had to be immobilized during irradiation procedure, so the sham-irradiated controls (C) were treated equally, except for being exposed to the source of radiation. The heads of the IR rats were exposed to a single 10 Gy dose of γ-rays using Co^{60}-source (Institute of Nuclear Sciences "Vinča"). The hippocampus was isolated at the following post-irradiation times: 1 h, 2 h, 4 h and 8 h. The levels of *p53* and *bax* mRNA were assessed by semi quantitative RT-PCR using as the internal standard mRNA for actin. The levels of p53 and Bax proteins in cytosolic fractions were detected by Western blot using mouse anti-p53 (StressGene) and rabbit anti-Bax antibody (Cell Signaling), respectively. β-Actin (Santa Cruz Biotechnologies) was used as an equal loading control. Analysis of variance (one-way ANOVA) followed by *post hoc* Tukey test was used to estimate statistically significant differences (P <0.05).

Results and discussion

It was well known that the tumor suppressor p53 protein is induced in response to irradiation and genotoxic drugs in order to produce either cell cycle arrest or apoptosis, depending on the cell type and the microenviroment [1]. Both of these events can be viewed as ways of protecting cells from DNA damaging agents. In this study, cranial irradiation rapidly stimulated p53 protein expression in a time-dependent manner (Figure 1A, B), whereas the level of *p53* mRNA expression showed no statistically significant changes (data not shown).

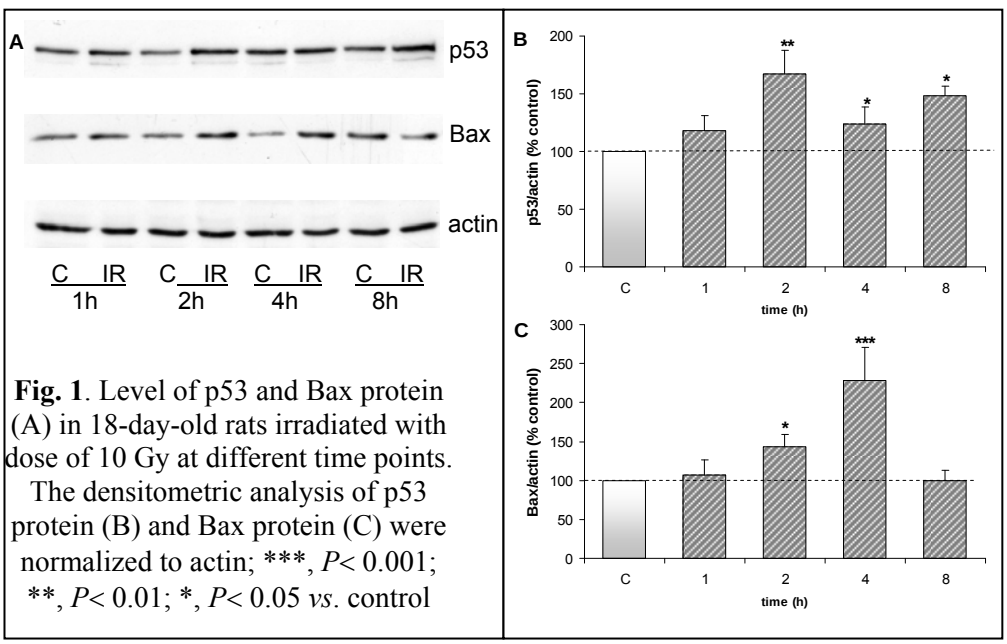

Fig. 1. Level of p53 and Bax protein (A) in 18-day-old rats irradiated with dose of 10 Gy at different time points. The densitometric analysis of p53 protein (B) and Bax protein (C) were normalized to actin; ***, P< 0.001; **, P< 0.01; *, P< 0.05 *vs*. control

These results are in agreement with a previous report, demonstrating that gamma-irradiation induces apoptosis as well as the induction of p53 protein in fetal rat brain, without a major up-regulation of *p53* mRNA [4].

It is known that p53 transcriptionally activates Bax in some types of cells after treatment with ionizing radiation, chemotherapeutic drugs and other forms of genotoxic stress [4]. We found that the level of cytosolic Bax protein was higher in irradiated rats that in the control ones; the maximum level was attained at 4 h, then subsequently reduced again to low level (Figure 1C). This induction temporally coincides with the elevation of p53 protein (Figure 1B), as expected for a cause-and-effect relationship. Moreover, cranial irradiation moderately increases the level of *bax* mRNA, as revealed by RT-PCR (Figure 2).

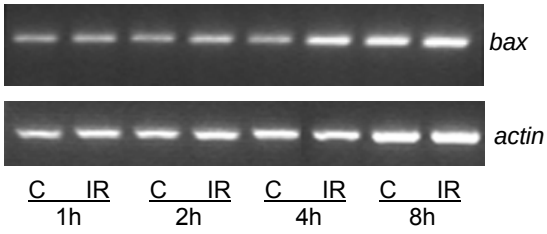

Fig. 2. Level of hippocampal *bax* mRNA expression in rats irradiated with 10 Gy at different time points.

Bax promotes apoptosis by facilitating release of apoptosis inducing factor and cytochrome-*c* from the mitochondria, thus triggering a cascade of caspase activation. It should be noted, however, that not all types of radiation-induced apoptosis involve p53 induction [5]. In irradiated p53-/- lymphoblasts overexpression of Bcl-2 protein leads to apoptosis, hence p53 is not the only mediator of apoptosis provoked by DNA damage [5].

Conclusion

Our result exemplifies the activation of p53-dependent genes by irradiation in this animal model system. Ionizing irradiation leads to rapid induction of p53 protein in the cytoplasm of rat hippocampal cells, without change in its mRNA level. This is followed by induction of positive apoptosis regulator Bax, both at the level of protein and mRNA.

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