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LOW DOSE GAMMA-IRRADIATION ALTERS THE EXPRESSION OF P53 PROTEIN IN THE RAT HIPPOCAMPUS

N. Veličković, A.D. Đorđević and N. Popović

Laboratory for Molecular Biology and Endocrinology, "Vinča" Institute of Nuclear Sciences, P.O. Box 522, Belgrade, Serbia

Abstract

The tumor-suppressor p53 protein and glucocorticoid receptor (GR) respond to different types of stress. In an attempt to reveal the possibility that p53 protein is involved in the regulation of GR gene expression after low dose cranial irradiation (CI) with 2 Gy, we examined the expression of p53 mRNA and protein as well as expression of GR mRNA and protein in the hippocampus of 18-days-old rats. We found that p53 mRNA expression was unchanged after CI, while induction of p53 protein was rapid, leading to the accumulation of p53 protein in the cytoplasm. Irradiation leads to stimulated GR gene expression in a time-dependent manner, whereas the level of GR protein was unchanged after CI. Co-immunoprecipitation has not showed that wild type p53 protein physically interacts with the GR in the cytoplasm. Our data suggest that the low dose cranial irradiation leads to stabilization of the 53 protein, without interaction with GR protein the cytoplasm.

Introduction

The tumor suppressor p53 gene encodes a nuclear phosphoprotein that functions as a key regulator of cell cycle arrest or apoptosis in response to various stresses, such as DNA damage, irradiation and hypoxia [1]. Glucocorticoid receptor (GR) is a member of the steroid receptor superfamily that mediates physiological processes controlled by glucocorticoids (GCs). The primary physiological function of GCs is to maintain homeostasis response to environmental changes [2]. Recently, evidence has been growing for cross-talk between the p53- and GR-mediated responses to stress. p53 physically interacts with and represses the activities of GR [3]. GCs prevent p53-induced apoptosis in human granulose cells [4]. Upon irradiation of cells with gamma-rays, the level of p53 protein is increased, presumably not followed with change in the mRNA level [5]. Our previous results showed the apparent induction of GR mRNA in the rat hippocampus after 10 Gy of gamma-irradiation. Because little is known about the functional interactions between signaling pathways mediated by GR and p53 under physiological conditions, we have investigated how p53 and GR regulate each other after low dose cranial irradiation (2 Gy) *in vivo* in rat hippocampus. The hippocampus was isolated at the following post-irradiation times: 1 h, 2 h, 4 h, 8 h, and 24 h. The level of GR and p53 mRNA was assessed by semi quantitative RT-PCR using as the internal standard mRNA for glyceraldehyde-6-phosphate dehydrogenase (GAPDH).

Results and Discussion

Low dose gamma radiation, routinely used in diagnostic protocols and clinical radiotherapy, have profound effects on the brain, leading to precursor cell dysfunction and debilitating cognitive decline. Although a plethora of molecular mechanisms are involved in acute effects of irradaition, one molecule is inevitable in decision between life and death: tumor-suppressor p53. Low dose irradiation rapidly stimulated p53 protein in a time-dependent manner (Figure 1), whereas the level of p53 mRNA expression showed no statistically significant changes (data not shown).

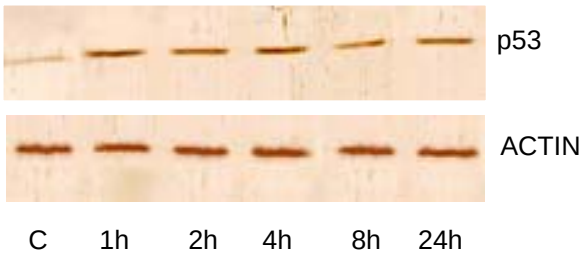

Fig. 1. Level of p53 protein expression in 18-days-old rats irradiated with dose of 2 Gy at different time points. The densitometric analysis of p53 protein were normalized to actin; C-immobilized control rats

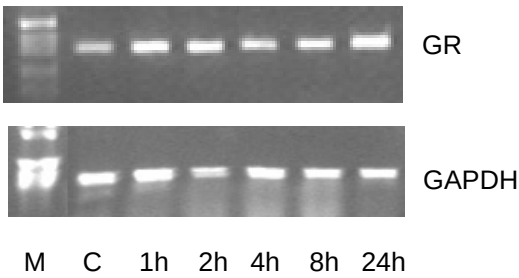

Fig. 2. Level of hippocampal GR mRNA expression in rats irradiated with 2 Gy at different time points. The densitometric analysis of GR mRNA were normalized to GAPDH M-DNA molecular weight marker C-immobilized control rats

Low dose CI (2 Gy) leads to induction of GR gene expression with peak expression observed after 2 h, remaining high up to at least 24 h (Figure 2). In contrast to GR mRNA, the dose of 2 Gy did not alter the level of GR protein as revealed by preliminary Western blot experiments (Figure 3).

On the other hand, in our previous experiments, irradiation with 10 Gy leads to cytoplasmatic sequestration of activated GR (after treatment with synthetic hormone dexamethasone).

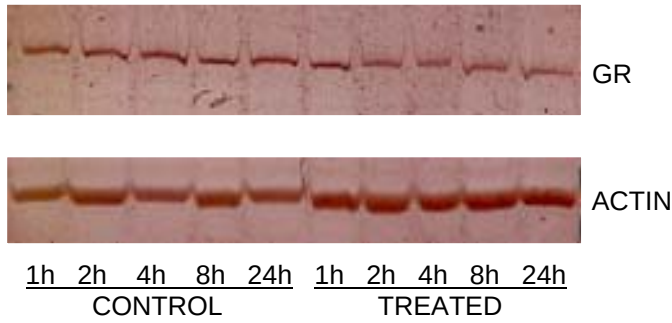

Fig. 3. Level of GR protein expression in 18-days-old rats irradiated with dose of 2 Gy at different time points. The densitometric analysis of GR protein were normalized to actin; Control-immobilized control rats

According to other publications, GR forms a complex with p53 *in vivo* in neuroblastoma cells thus resulting in cytoplasmatic retention and inactivation of wild type p53 [3]. Our study has not showed that wild type p53 protein physically interacts with the GR in cytoplasm.

Conclusion

Our results showed that low dose gamma irradiation leads to rapid induction of p53 protein in the cytoplasm of rat hippocampal cells, without change in mRNA level. On the contrary, the GR mRNA was increased after irradiation with 2 Gy, while the level of GR protein was unchanged. Co-immunoprecipitation showed that the accumulation of p53 protein in the cytoplasm was not a result of physical interaction of GR and p53 protein. These data may contribute to unraveling the mechanism of antiapoptotic effects of GCs, since dexamethasone is frequently used as a co-treatment agent in cancer therapy.

Acknowledgement

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