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KINETICS OF DSB INDUCTION AND CHANGES IN CELL CYCLE REGULATION IN MELANOMA CELLS AFTER IONIZING RADIATION

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Abstract
The effects of γ-rays on the DNA level, i.e. formation of double-strand breaks and expression of p21 were studied *in vitro* on the human HTB 140 melanoma cells. Cells were exposed to the dose range from 2 to 16 Gy. Effects were analyzed 30 min, 2, 6 and 24 h after irradiation. It has been shown that the level of phosphorylated histone H2AX (γH2AX) is time- and dose-dependent, as well as the expression of p21.

Introduction
Malignant melanoma is a very aggressive type of cancer, generally resistant to different therapeutic approaches. One of the reasons for such behavior is its significant ability to repair DNA breaks [1]. Ionizing radiation (IR) acts on the DNA, either directly by inflicting DNA damages, or indirectly by affecting DNA metabolism and causing damages. As a result, cell cycle checkpoint activation becomes an important determinant of the ultimate response to the treatment. This will benefit existing therapeutic modalities and likely contribute to the development of novel cancer-treatment approaches. Extensive studies uncovered a complex network of genes that cooperate to delay the normal progression through the cell cycle as soon as the damage is registered in the genome [2]. When human cells sustain a DNA double-strand break (DSB), histone H2AX in chromatin surrounding the DNA break becomes phosphorylated at serine 139 (γH2AX), thus making repair foci [1]. Literature data suggest a correlation between radio-sensitivity and the kinetics of γH2AX clearance in human tumor cell lines. Radio-sensitive tumor cells retain γH2AX for a greater duration than radio-resistant ones [3].

The p21 (CIP1/WAF1) protein binds to and inhibits the activity of cyclin-CDK2 or -CDK1 complexes, and therefore functions as a regulator of cell cycle progression at G1. Also, p21 can lead to G2 arrest. H2AX phosphorylation has been shown to be required to maintain p21 level, leading to cell cycle arrest [4].

The aim of this study was to investigate the kinetics of DSB induction in human HTB140 melanoma cells after exposure to γ-rays. This was done through evaluation of the level of γH2AX and the expression of p21 protein.

Results and Discussion

Irradiations of HTB140 cells with γ-rays were performed using ^{60}Co source, at the Vinča Institute of Nuclear Science in Belgrade. The dose range was from 2 to 16 Gy. The average dose rate was ~1 Gy/min. All cell irradiations were carried out in air at ~0°C.

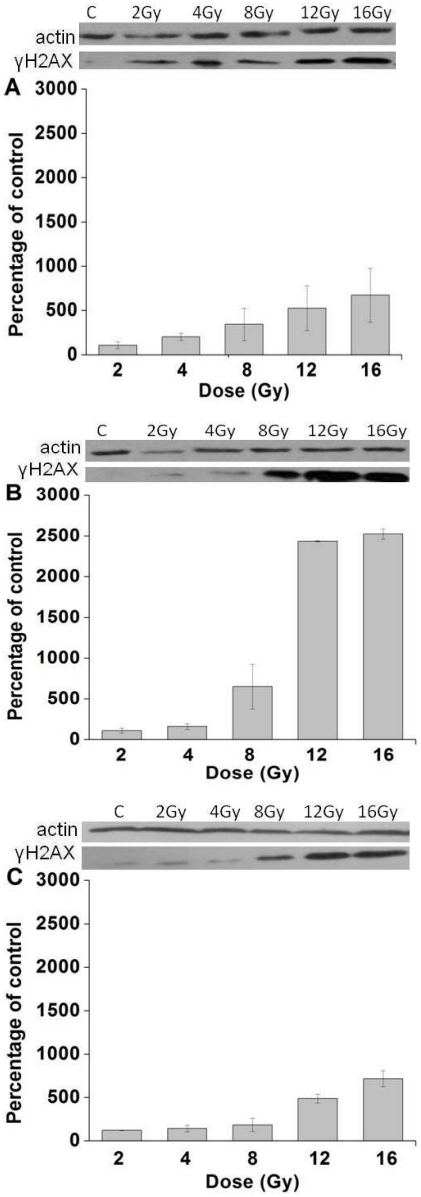

Figure 1. Level of γH2AX in HTB140 cells analyzed by Western blot, 30 min (A), 2 h (B) and 6 h (C) after irradiation. Results are presented as percentage of control (mean ± S.D.). C-control.

After irradiations, cells were incubated for 30 min, 2, 6 and 24 h under standard conditions. The levels of H2AX and p21 were analyzed by Western blot. Phosphorylation of H2AX has reached the highest value at 2 h after irradiation (Fig. 1). At 6 h, as well as after 24 h post-irradiation (data not shown), there is a tendency of reduction of H2AX to the pre-irradiation level. This indicates a high level of radio-resistance of the HTB140 cells. Dose dependence is seen in all analyzed time points (Fig. 1A, B, C).

After inhibition of replication H2AX is required for the increase of p21 level, subsequently resulting in checkpoint activation and cell cycle arrest [4]. Consequently, the expression of p21 was analyzed. Obtained results showed that -rays induced dose dependent expression of p21 with the highest level attained at 6 h post-irradiation (Fig. 2B).

This implies that the p21 expression increases when the level of H2AX drops down. Since p21 is involved in the regulation of G1, as well as G2 arrest, these data are in accordance with our previously reported G2 arrest of the HTB140 cells exposed to radiation [5].

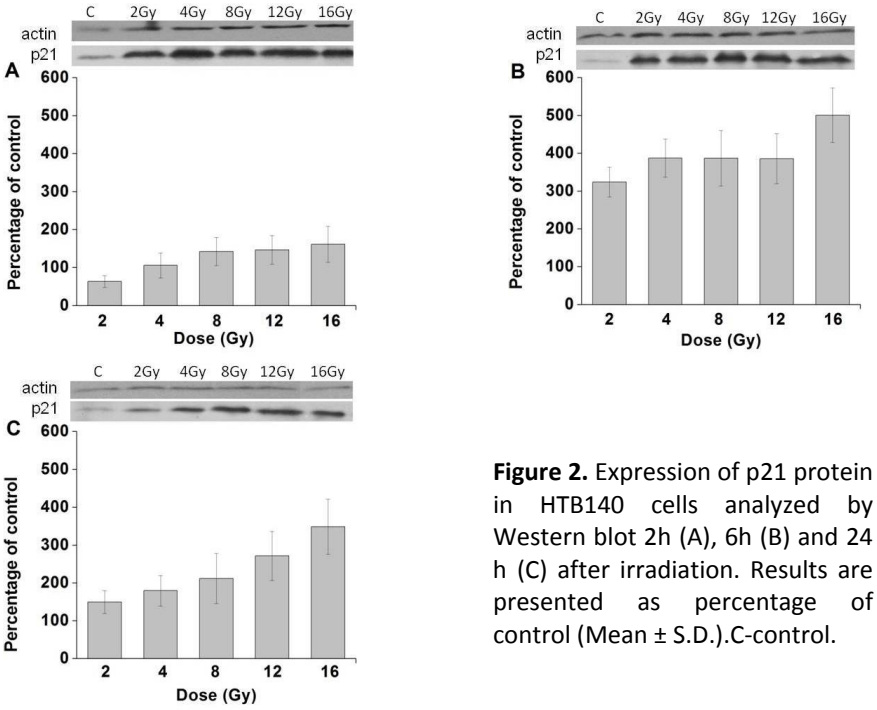

Figure 2. Expression of p21 protein in HTB140 cells analyzed by Western blot 2h (A), 6h (B) and 24 h (C) after irradiation. Results are presented as percentage of control (Mean ± S.D.).C-control.

Conclusions

Obtained results showed that γ-rays induced time- and dose-dependent phosphorylation of histone H2AX and the expression of p21 protein, indicating that this anti-tumor agent provokes induction of DSBs and changes in the cell cycle regulation in HTB140 cells. Kinetic of γH2AX loss confirmed radio-resistant nature of the analyzed cells. These findings give useful information about mechanisms of melanoma resistance, since γH2AX is used as a novel tool for monitoring genotoxic events associated with cancer.

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