**Analysis of cell response to ultrahigh dose-rate proton irradiation**

S.V.Akulinichev1,2, S.I.Glukhov3, E.A.Kuznetsova3, V.V.Martynova1, I.A.Yakovlev1,2

*1Institute for Nuclear Research RAS, Troitsk, RF;*

*2Hospital of the Russian Academy of Sciences, Troitsk, RF;*

*3Institute of Theoretical and Experimental Biophysics RAS, Pushchino, RF.*

E-mail: akulinic@gmail.com

 As is known, the flash effect in radiotherapy with a dose rate of more than 40 Gy/s makes it possible to destroy tumor cells much stronger than normal cells. Additional prospects for flash therapy are associated with irradiation with protons, since protons and ions make it possible to increase the conformality of irradiation compared to irradiation with light particles. A unique feature of the proton beam facility at the Institute of Nuclear Research [1], is that it makes it possible to deliver the total irradiation dose in one pulse with a duration of less than 100 μs. Such a single-pulse flash mode (splash) of radiotherapy may open up additional prospects.

 We have carried out a series of several runs of the INR proton accelerator in a wide range of modes: from the conventional mode with an average dose rate Ḋ < 3 Gy/s to the splash mode with Ḋ > 104 Gy/s. Two types of tumor cells were irradiated in these experiments: human colon adenocarcinoma (HT-29) and human colon cancer (HCT116). Human adipose tissue mesenchymal stem cells (ADSC) – fibroblasts – were taken as normal cells. Cell cultures were irradiated in the region of the Bragg peak (SOBP) and on the plateau up to the Bragg peak. The task is to carry out a comprehensive analysis of the cell response to various modes of proton irradiation, both using flow cytometry and using another method - real-time PCR. Quantitative PCR was used to analyze the genetic control of apoptosis initiation (BAX, PUMA genes), cell cycle control (CDKN1A gene), and genome integrity control (p53 gene).

 According to preliminary results, the levels of expression of genes involved in apoptosis and genome integrity control under flash/splash irradiation differ from those under conventional irradiation both in the studied tumor lines and in normal fibroblasts.

 The work is supported by the Russian Science Foundation grant No. 22-25-00211 “Investigation of cell response to the impact of record powerful ultrashort proton pulses.”

1. Akulinichev S.V., et al. Possibilities of Proton FLASH Therapy on the Accelerator at the Russian Academy of Sciences’ Institute for Nuclear Research. Bull. Russ. Acad. sci. Phys. 84, 1325–1329 (2020).