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The Activation of DNA Repair Pathways after Ultra-Short Pulsed Electron Beam Irradiation in Human Cells

(Submitted by corresponding member of NAS RA R. M. Aroutiounian 2/V 2022)

Keywords: *irradiation; accelerators; ultrashort pulsed electron beam; DNA repair; K-562 cell line.*

Introduction. A new generation of accelerators is actively developing in contemporary radiotherapy. Laser-generated accelerators produce ultra-short electron beam pulses, which provide a unique opportunity to generate new molecular genetics effects [1-3]. Due to the high-frequency laser source, the AREAL (Advanced Research Electron Accelerator Laboratory, Armenia) accelerator gives ultra-short and high-speed electron flux producing ultra-short pulsed electron beam (USPEB) radiation [4]. The study of the genetic effects of laser-generated USPEB radiation is the relevant direction in radiobiology.

It was shown that USPEB radiation-induced DNA DSBs are characterized by slow elimination kinetics. The low level of micronuclei frequency and apoptosis was evident in human lung fibroblasts irradiated by USPEB [5]. An assessment of the dependence of the radiation effect on sex showed that females PBMCs had higher sensitivity to irradiation, as the DNA damage level was 4 times higher compared to the control, while in males the increase was less than 1.5 times [6]. Ionizing radiation (IR) mainly induces single-strand breaks (SSBs), double-strand breaks (DSBs), modified bases, mismatches, and basic sites [7]. The DNA repair can have alternative mechanisms. The homologous recombinational repair (HRR) works by exchanging similar but non-identical DNA between homologous chromosomes. Non-homologous end joining (NHEJ) works by inserting or deleting genes from a broken site, besides it is faster, but also is an error-prone mechanism [8]. DNA-dependent protein kinase (DNA-PKcs) is one of the significant components of the NHEJ repair pathway, and is a catalytic subunit of DNA-PKcs promoting synapsis of broken DNA ends, with further stages of end-processing and ligation [9]. On the contrary, the HR repair pathway is slower but with fewer errors in repaired strands. The

MRE11 nuclease plays a crucial role in the HR repair pathway, initiating double-strand breaks (DSB) resection [10]. On the other hand, the accumulation of single-strand breaks (SSBs) also brings to DSBs formation, and the main repair pathway of SSBs is Base Excision Repair (BER). The APEX endonuclease is a DNA repair enzyme with apurinic/apyrimidinic (AP) activity in the DNA BER pathway [11].

This study aimed to evaluate the USPEB irradiation-induced DNA DSBs and SSBs repair mechanisms in human K-562 (chronic myelogenous leukemia) cells.

Materials and Methods. ***Cell culture.*** The K-562 (human chronic myeloid leukemia) cell line was maintained in RPMI-1640 (Sigma Aldrich, Germany), supplemented with 10% Fetal Bovine Serum (HyClone, UK), 2 mM L-glutamine (Sigma Aldrich, Germany), 100 IU/ml penicillin (Sigma Aldrich, Germany) and 100 μg/ml streptomycin (Sigma Aldrich, Germany) at 37°C, 5%CO_2. Prior to irradiation, 2 ml of cells seeded in Eppendorf tubes (Sigma Aldrich, Germany) at a density of 0.5×10^5 cells/ml.

Irradiation. Radiation treatment was carried out using an electron beam generated by a laser-driven radiofrequency gun-based linear AREAL accelerator. For cell irradiation, each sample was placed in a sample holder facing towards the horizontal beam coming from the direction of the vacuum window. Cell samples were placed horizontally at the center of a 1 cm × 1 cm area at 1 cm from the beam exit point of the accelerator. Cells were irradiated with doses of 0.5, 4 and 8Gy, non-irradiated cell cultures were used as a control.

Enzyme-Linked Immunosorbent Assay (ELISA Indirect). The DNA repair pathways activation was analyzed using Colorimetric Cell-Based ELISA kits (Assay Biotech, USA) following the manufacturer instructions. After irradiation, the cells were seeded into 96 well plate and incubated overnight at 370C, 5%CO2. Cells were washed with 200 µl of 1x TBS (Tris-buffered saline) twice, fixed with 100 µl of Fixing Solution, and set for 20 minutes at room temperature. After incubation, 100 µl Quenching Buffer was added and set for 20 minutes at room temperature. After 200 µl of Blocking Buffer was added and incubated for 1 hour at room temperature. Then 50 µl of 1x primary antibodies were added to the corresponding wells and incubated overnight at 4^0C. After, added 50 µl of 1x secondary antibodies were added to corresponding wells and incubated for 1.5 hours at room temperature. The 50 µl of Ready-To-Use Substrate was added to each well and incubated for 30minutes at room temperature in the dark, and 50 µl of Stop Solution was added to each well and the OD was measured at 450nm using ELISA plate reader HumaReader HS (Human, Germany).

Statistical analysis. Statistical analyses of the data were conducted using the Statistica 8.0 software (StatSoft, Tulsa, OK, USA). The results are presented as the means of three independent experiments ± standard error. The non-parametric Mann–Whitney test was used for statistical analysis and $P<0.05$ was considered to indicate a statistically significant value.

Results and discussion. The activation kinetics of DNA DSBs and SSBs

repair pathways after ultrashort pulsed electron beam irradiation was studied in the K562 cell line. Radiation doses were considered as non-lethal at the dose of 0.5Gy (cell survival after 24h was 90%), sub-lethal at the dose of 4Gy (cell survival after 24h was 50%), and lethal at the dose of 8Gy (cell survival after 24h was 10-20%). The DNA repair kinetics of irradiated samples was assessed at 0h, 30min, 1h, and 4h time points after irradiation. The activation of HR, NHEJ and BER DNA repair pathways was detected. The 0h time-point was selected to assess the primary or background DNA damage reparation machinery activation.

In case of irradiation at the non-lethal dose (0.5Gy) the level of repair proteins DNA-PK, MRE11, and APEX1, responsible for appropriate repair pathways, was estimated immediately after irradiation (at the 0h time point), and the expression of MRE11 was more pronounced (Fig. 1). At the 30min time point after irradiation the level of DNA-PK and MRE11 proteins increased significantly, while the level of BER protein remained unchanged. At the 1h and 4h time point after irradiation the increase of the BER protein expression was observed, while the level of DNA-PK and MRE11 proteins decreased and eventually all proteins reached the same level at the 4h time point.

In the case of irradiation at the sub-lethal dose (4Gy), the level of DNA-PK, MRE11, and APEX1 proteins was assessed at the 0h time point where the expression DNA-PK was more enunciated (Fig. 2). At the 30min time point after irradiation, the level of DNA-PK and MRE11 proteins increased noticeably, while the level of APEX1 protein reduced. At the 1h time point after irradiation, the levels of proteins were nearly the same as in 30min. At the 4h time point after irradiation, all 3 protein expressions were increased, and the DNA-PK, MRE11, and APEX1 proteins reached the peck level.

After the irradiation at the lethal dose (8Gy), the level of repair proteins DNA-PK, MRE11, and APEX1, at the 0h time point was the same (Fig. 3). At the 30min time point after irradiation, the level of DNA-PK, MRE11, and APEX1 proteins increased, reaching a peak level. At the 1h and 4h time points after irradiation, the decrease of the expression of the protein was observed, which reduced up to the control level.

It can be assumed, that cancer cells recognize the level of DNA damages (non-lethal, sub-lethal, and lethal) and initiate the different repair pathway machinery. In case of non-lethal level of damages, cancer cells effectively repair DNA DSBs, and the error-prone NHEJ pathway prevails in this case, while SSBs reparation remains postponed. It can be explained by the nature of cancerous cells, which tend to accumulate minor damages to maintain the genome instability. However, at the higher doses of irradiation cancer cells activate the whole pull of repair capacity ensures their viability.

Conclusion. It was shown that ultrashort pulsed electron irradiation simultaneously activates HR and NHEJ repair systems in K-562 cells 30 minutes after irradiation, including BER at the 8Gy of irradiation dose. After 1h and 4h of irradiation, the level of expression of repair systems decreases at 0.5Gy and 8Gy doses of radiation, except the activity of BER system, which

remains high at 4h after irradiation at the dose of 0.5Gy. At the irradiation dose of 4Gy, the dynamics of an increase in the activity of all three repair systems was observed up to 4h after irradiation. Thus, the dynamics of changes in repair activity depend on the radiation dose.

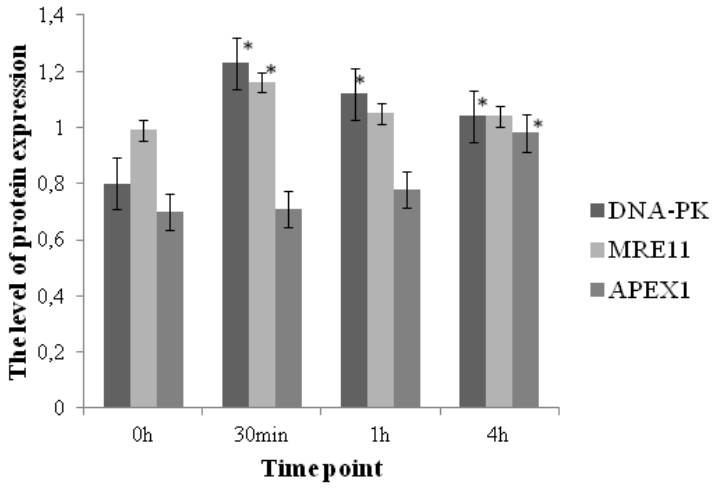

Fig. 1. The level of DNA-PK, MRE11, APEX1 repair proteins, after USPEB irradiation at the 0.5Gy dose.*P<0.05.

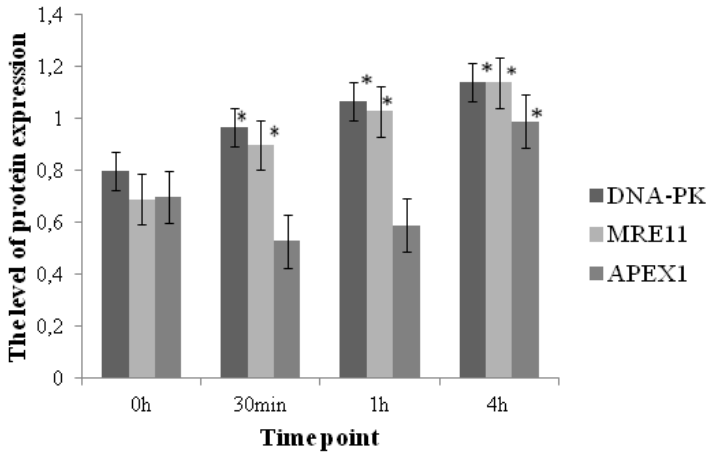

Fig. 2. The level of DNA-PK, MRE11, APEX1 repair proteins, after USPEB irradiation at the 4Gy dose. *P<0.05.

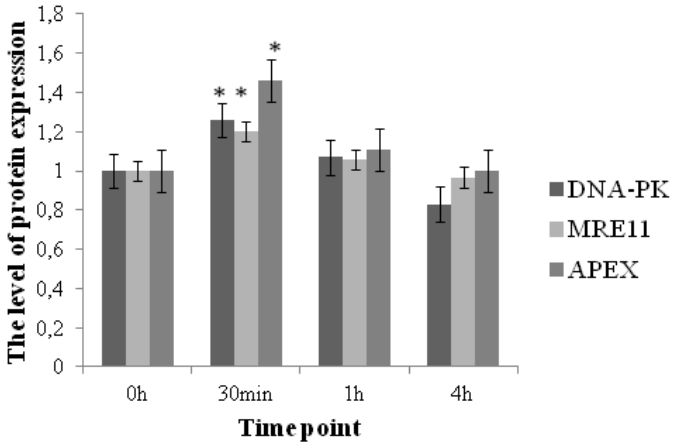

Fig. 3. The level of DNA-PK, MRE11, APEX1 repair proteins, after USPEB irradiation at the 8Gy dose. *P<0.05.

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The Activation of DNA Repair Pathways after Ultra-Short Pulsed Electron Beam Irradiation in Human Cells

The aim of this work was to estimate the differences in activation of DNA repair pathways of DNA double-strand and single-strand breaks induced by ultrashort pulse electron beam irradiation in human K-562 cells. The activation of HR, NHEJ and BER DNA repair pathways was studied at non-lethal, sub-lethal and lethal doses of irradiation. Our results indicate that the activation of the specific repair pathways and repair kinetics depend on the irradiation dose.

Ա. Թ. Մանուկյան

Գերկարճ իմպուլսային էլեկտրոնային ճառագայթմամբ խթանված ԴՆԹ ռեպարացիոն ուղիների ակտիվացումը մարդու բջիջներում

Գնահատական է տրվել գերկարճ իմպուլսային էլեկտրոնային ճառագայթմամբ մականված ԴՆԹ-ի միաշղթա և երկշղթա վնասվածքների ռեպարացիոն ուղիների ակտիվացման օրինաչափությանը մարդու K-562 բջիջներում: Ուսումնասիրվել է ԴՆԹ-ի հոմոլոգիական, ոչ հոմոլոգ ծայրերի միացման և հիմքի էքսցիզիոն ռեպարացիոն

ուղիների ակտիվացումը ճառագայթման ոչ լետալ, սուբ-լետալ և լետալ չափաբաժիններով ճառագայթման պայմաններում: Ըստ ստացված արդյունքների՝ ռեպարացիոն որոշակի ուղու ակտիվացումը և վերականգնման կինետիկան կախված են ճառագայթման չափաբաժնից:

А. Т. Манукян

Активация репарационных путей ДНК в клетках человека при ультракоротком импульсном электронном облучении

Дана оценка активации репарационных путей ДНК при двуцепочечных и одноцепочечных разрывах, индуцированных ультракоротким импульсным электронным облучением в К-562 клетках. Исследованы репарационные пути гомологичной рекомбинации, негомологичного соединения концов и эксцизионной репарации оснований при нелетальной, сублетальной и летальной дозах облучения. Показано, что активация специфического пути репарации и кинетика репарации зависят от дозы облучения.

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