DNA DOUBLE STRAND BREAKS INDUCED BY ULTRASHORT PULSED ELECTRON BEAM IRRADIATION IN HUMAN BLOOD CANCER AND NORMAL CELLS

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Аннотация: Быстро развивающиеся лазерные технологии положили начало эволюции ускорителей частиц, генерируемых лазером, в качестве альтернативы существующему оборудованию. Целью данной работы было изучение формирования и репарации белка-маркера двунитевых разрывов ДНК фосфорилированного гистона (γH2AX) в культуре клеток хронического миелоидного лейкоза (K562) и нормальных мононуклеарных клетках периферической крови (МКПК) человека после облучения ультракоротким импульсным электронным пучком методом проточной цитометрии. Результаты исследований показали что, уровень образования двунитевых разрывов ДНК и кинетика репарации отличаются в опухолевых K562 и нормальных МКПК клетках.

Ключевые слова: облучение; лазерные ускорители; АРЕАЛ; ультракороткий импульсный электронный пучок; проточная цитометрия; γH2AX; K562; МКПК.

Abstract: Quickly developing laser technologies have started the evolution of laser-generated particle accelerators as an alternative to current equipment. The aim of this work was to analyze the formation and repair of phosphorylated histone γH2AX as a marker of DNA double strand breaks by flow cytometry in human chronic myeloid leukemia (K562) and normal peripheral blood mononuclear cells (PBMCs) in response to ultrashort pulsed electron beam irradiation. Our results indicated that DNA DSBs formation level and repair kinetics was different in cancerous K562 and normal PBMC cells.

Keywords: irradiation; laser-driven accelerators; AREAL; ultrashort pulsed electron beam; flow cytometry; γH2AX; K562; PBMC.

Introduction

In the field of modern radiotherapy, the creation and development of a new generation of accelerators is actively developing, which opens up new avenue in increasing the effectiveness of cancer radiotherapy. Laser-driven particle beams generated by linear AREAL accelerator at the "CANDLE" Synchrotron Research Institute (Armenia) are characterized by ultrashort duration (femtoseconds), high peak dose-rate (up to tens of Gy/s during pulse), monoenergetic spectral profile and low side divergence [1]. It is known that the DNA double-strand breaks (DSBs) are the most harmful lesions induced by ionizing radiation (IR), and the phosphorylated histone H2AX (γH2AX) is a marker of DSBs formation after irradiation [2].The aim of this work was to study the formation and repair kinetics of γH2AX as a marker of DSBs in human blood cancer (K562) and normal peripheral blood mononuclear (PBMCs) cells in response to ultrashort pulsed electron beam generated by AREAL accelerator.

Materials and Methods

PBMCs isolation and cell culture

Blood samples were collected by venipuncture from healthy donors. PBMCs were isolated using Ficoll-Paque (Sigma Aldrich, Germany) density centrifugation. Both the K562 (chronic myelogenous leukemia) and PBMCs were cultivated in RPMI-1640 (Sigma Aldrich, Germany), supplemented with 10% Fetal Bovine Serum (HyClone, UK), 2mM 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 at the density of 0.5×10^5 cells/ml was transferred to the Eppendorf tubes (Sigma Aldrich, Germany).

Irradiation

Irradiation was carried out using an electron beam generated by a laser-driven radiofrequency gun-based linear AREAL accelerator [1]. The beam main parameters are: beam charge-30 pC, electron energy-3.6 MeV, pulse duration-450fs, pulse repetition rate-20 Hz, beam spot size-15mm, RMS energy spread<1.15%, online dosimetry was performed by Faraday cup. Cells were irradiated at doses of 4 and 8Gy, with a repetition rate of 20 Hz. The mean absorbed dose-rate of 11.70 ± 0.98

Gy/min was calculated over the period of irradiation and 1% charge fluctuation and 1% beam energy fluctuation was taken into account.

γH2AX assay by Flow cytometry

30 min, 1, 4 and 24h post-irradiation, the cells were fixed in fixative solution (BD Biosciences, 554655) for 30min at 4°C, followed by permeabilization with Perm Buffer III(BD Biosciences, 558050) and washing with stain buffer (BD Bioscience, 554656). Afterwards, the cells were incubated with γH2AX - PE antibody (BD Biosciences, 552377) for 30 min in dark. Stained samples were analyzed on BD FACScan flow cytometer (Becton Dickinson, San Jose, CA) and 10000 non-debris events were obtained. The data was analyzed using CellQuest Pro (Becton Dickinson San Jose, CA) software.

Statistical analysis

Data analysis was performed by Graph Pad Prism 5.01 (Graph Pad Software, USA).The results are presented as the means ± standard error (SE). Statistical significance was tested by nonparametric Kruskal-Wallis with Dunn's multiple comparisons test. A $p<0.05$ was considered as statistically significant.

Results and discussion

The results of DNA DSBs formation and repair kinetics in human blood cancerous and normal cells after ultrashort pulsed electron beam irradiation are presented in Fig. 1. The overall level of DNA damages in human PBMCs was higher as compared to the K562 cells at all doses of irradiation and time points. In case of cancerous K562 cells the peak level of γH2AX formation was observed 1h-post irradiation at the doses of 4Gy and 8Gy. After 4h and 24 h of exposure the reparation activity was evident in K562 cells, however the level of DNA DSBs after 24 h was significantly higher than that of 30 min post-irradiation (Fig. 1a).

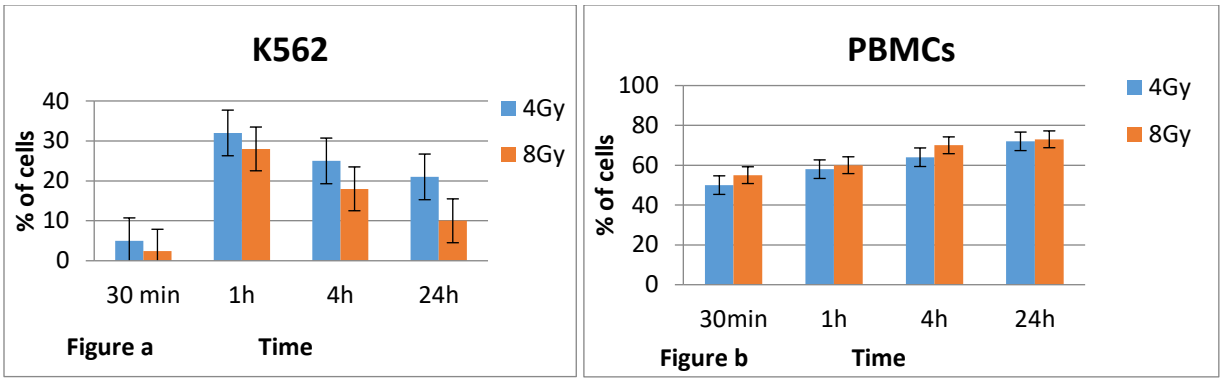

Figure 1. The kinetics of γ-H2AX foci formation in human cancer (a) and normal (b) cells.

The percentage of cells with higher number of γH2AX was shown in case of irradiation at the dose of 4Gy as compared to 8Gy irradiation, which can be attributed to the death of cells with highly damaged DNA after lethal dose irradiation (8Gy). In case of PBMCs, the increase in the percentage of cells expressing γH2AX was observed at both doses and all studied time points, reaching 73% at 24h post-irradiation (Fig. 1b). Thus, these results may indicate inhibition of the reparation activity in normal blood cells.

Conclusion

The level of DNA DSBs formation and repair kinetics are differ in human blood cancerous K562 and normal PBMC cells at sub-lethal and lethal doses of irradiation with ultrashort pulsed electron beam irradiation. In K562 cells the reparation activity is observed at 4h and 24 h time points, while in PBMCs the increased level of DNA DSBs in dose and time-dependent manner was evidenced.

References

1. Tsakanov V.; Aroutiounian R.; Amatuni G.; Aloyan L.; Aslanyan L.; Avagyan V.; Babayan N.; et al.// AREAL low energy electron beam applications in life and materials sciences.//Nucl. Instrum. Methods Phys. Res. A.// 2016; 829: 248–253.// doi.org/10.1016/j.nima.2016.02.028
2. Ulyanenko S.; Pustovalova M.; Koryakin S.; Beketov E.; Lychagin A.; Ulyanenko L.; Kaprin A.; Grekhova, A.M.; Ozerova, A.V.; Ozerov, I.; et al.// Formation of γH2AX and pATM Foci in Human Mesenchymal Stem Cells Exposed to Low Dose-Rate Gamma-Radiation. // Int. J. Mol. Sci. 2019, 20, 2645. // doi: 10.3390/ijms20112645