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Radiobiological research at Burnasyan Federal Medical Biophysical Center: focus on DNA double-strand breaks

Andreyan N. Osipov

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State Research Center - Burnasyan Federal Medical Biophysical Center of Federal Medical Biological Agency is the flagship institution of Russian healthcare in the field of radiobiology, radiation medicine and radiation protection.

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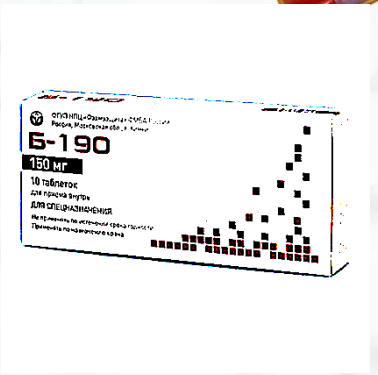
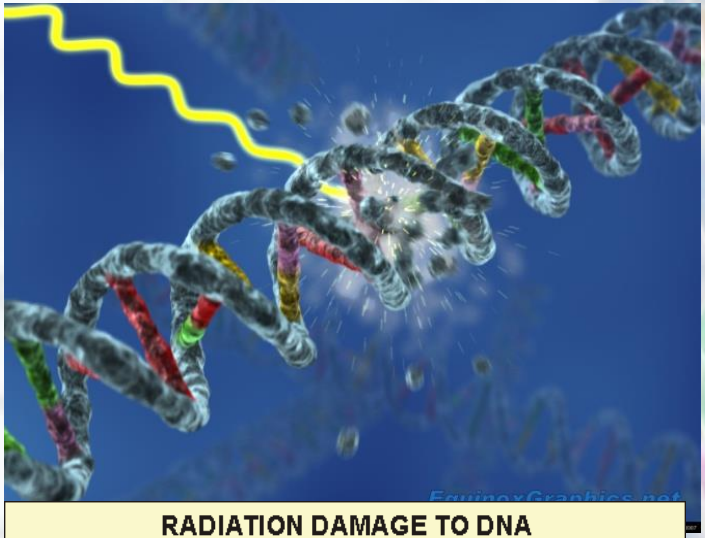


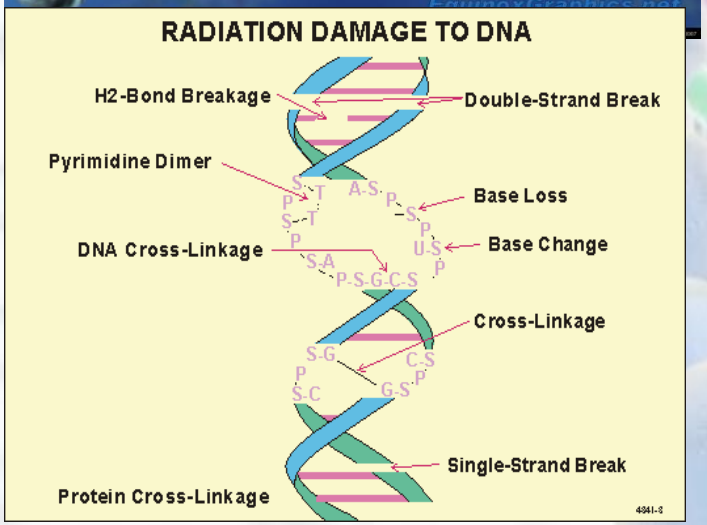
The main directions of radiobiological research activities:

- **Development of radioprotective drugs, methods and approaches for the prevention and treatment of radiation injuries.**

- **Study of molecular and cellular mechanisms of early and delayed effects of exposure to ionizing radiation.**

- **Development of diagnostic and prognostic criteria and biomarkers of radiation injury for the different tasks of radiation medicine.**





Yields of DNA Damage produced in 1 cell by 1 Gray

~ 1,000 single strand breaks

~ 3,000 damaged bases

~ 25-40 double strand breaks

~ 190 multiply damaged sites

- The majority of cellular DNA lesions caused by ionizing radiation (IR) significantly differ from those caused by endogenous sources in their physical and chemical properties.
- The most important features of radiation-induced DNA lesions are their complexity and clustering.
- Among the various types of primary DNA lesions produced by ionizing radiation, DNA double-strand breaks are of the most biological relevance as relates to radiological risks due to their high potential to cause cell death, mutagenesis and carcinogenesis.

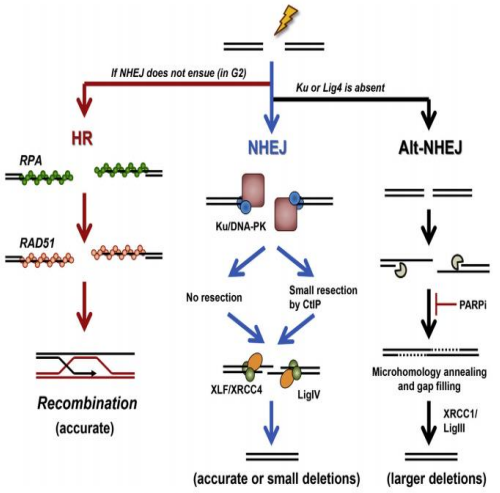


Fig 1. Pathways of double-strand break (DSB) rejoining and their hierarchy. Non-homologous end-joining (NHEJ) is the first choice DSB repair pathway in mammalian cells. However, pathways exploiting resection can be used if rapid repair by NHEJ does not ensue. Homologous recombination can be used in late S/G2 cells. Inaccurate NHEJ can also arise. Alternative NHEJ is predominantly only used when Ku or NHEJ proteins are absent.

A. Shibata, P.A. Jeggo / Clinical Oncology 26 (2014) 243–249

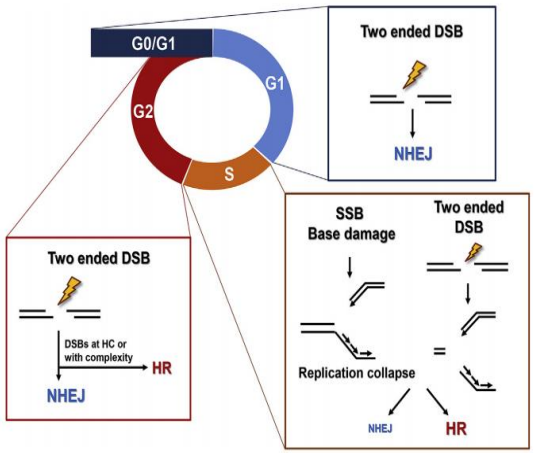
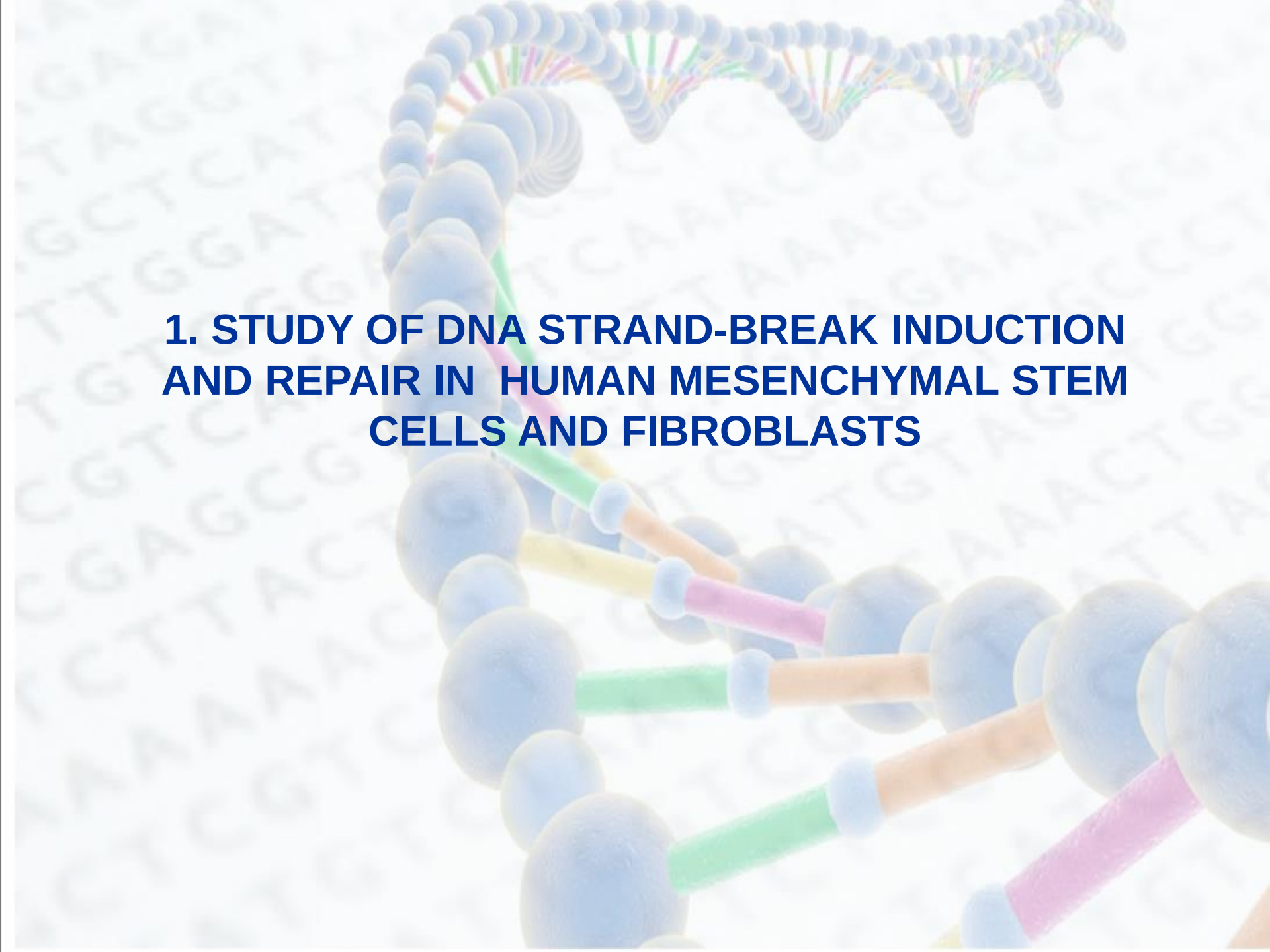


Fig 2. Cell cycle regulation of non-homologous end-joining (NHEJ) and homologous recombination. NHEJ can function in all cell cycle phases. Homologous recombination has a major role promoting recovery from replication fork stalling in S phase but can also function to repair radiation-induced two-ended double-strand breaks (DSBs) in late S/G2 phase using a sister chromatid. However, even in G2 phase, NHEJ is the major DSB repair pathway.

A. Shibata, P.A. Jeggo / Clinical Oncology 26 (2014) 243–249

- DNA double-strand breaks can be repaired by one of the two major mechanisms: non-homologous endjoining (NHEJ) or homologous recombination (HR). NHEJ repair, involved in an estimated ~ 70-80% of DNA double-strand breaks, is cell cycle independent and fast, taking approximately 30 minutes to complete. However, NHEJ is error-prone and can lead to various genetic abnormalities. In contrast, HR repair is errorfree and slow (> 7 h) and requires a sister chromatid as a template for DNA synthesis in the vicinity of a break on the damaged chromatid. Therefore, this pathway is active mainly in cells in S and G2 cell cycle phases.

1. STUDY OF DNA STRAND-BREAK INDUCTION AND REPAIR IN HUMAN MESENCHYMAL STEM CELLS AND FIBROBLASTS



Low doses of X-rays induce prolonged and ATM-independent persistence of γH2AX foci in human gingival mesenchymal stem cells

Andreyan N. Osipov[1,2,3,4], Margarita Pustovalova[1,2], Anna Grekhova[1,5], Petr Eremin[1], Natalia Vorobyova[1,3], Andrey Pulin[1], Alex Zhavoronkov[4,6,7], Sergey Roumiantsev[3,4,8], Dmitry Y. Klokov[9], Ilya Eremin[1]

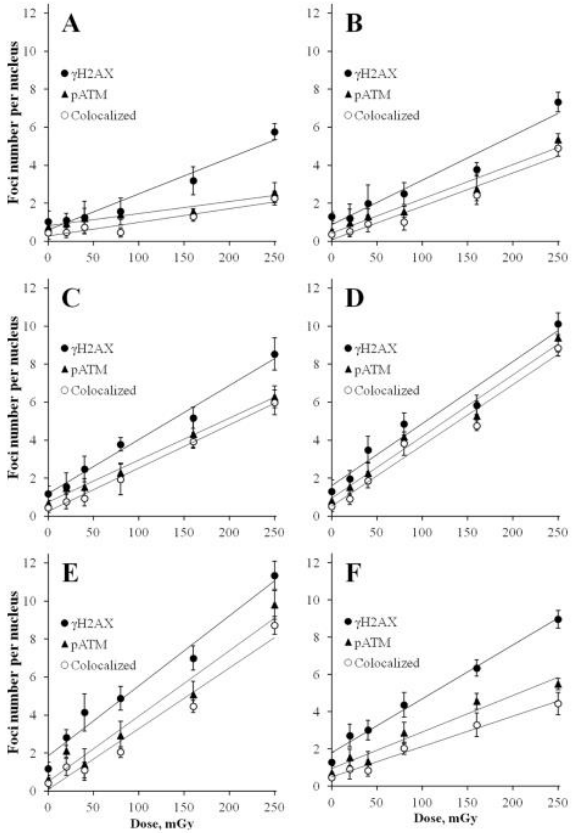


Figure 1: Radiation dose-responses for γH2AX and pATM foci in MSCs. Cells were exposed to X-irradiation at various indicated doses and fixed at 5 min **(A)** 10 min **(B)** 15 min **(C)** 30 min **(D)** 60 min **(E)** and 120 min **(F).** Immunofluorescence labeling for γH2AX and pATM was performed as described in Materials and Methods. Number of foci for each protein and the number of co-localized foci were quantified and mean values of three independent experiments ± SD are shown on the graphs.

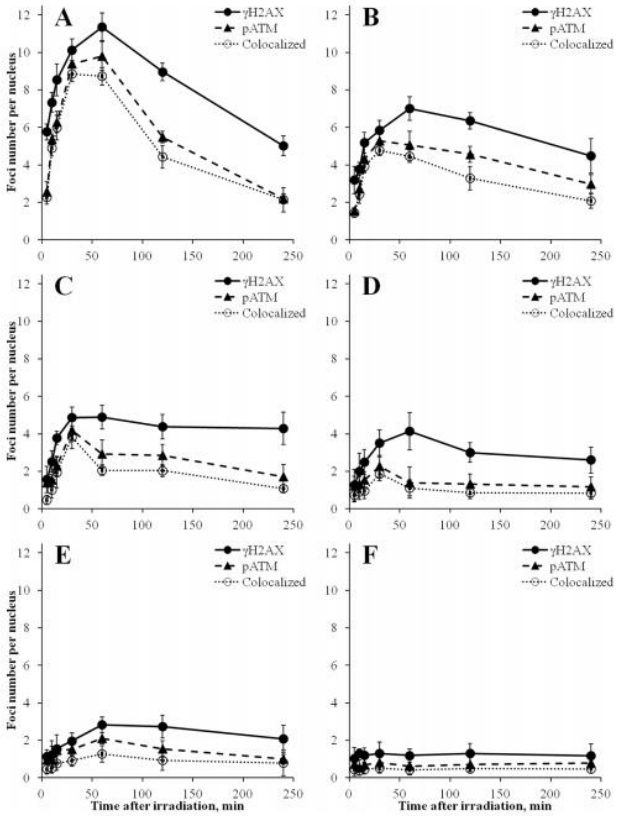
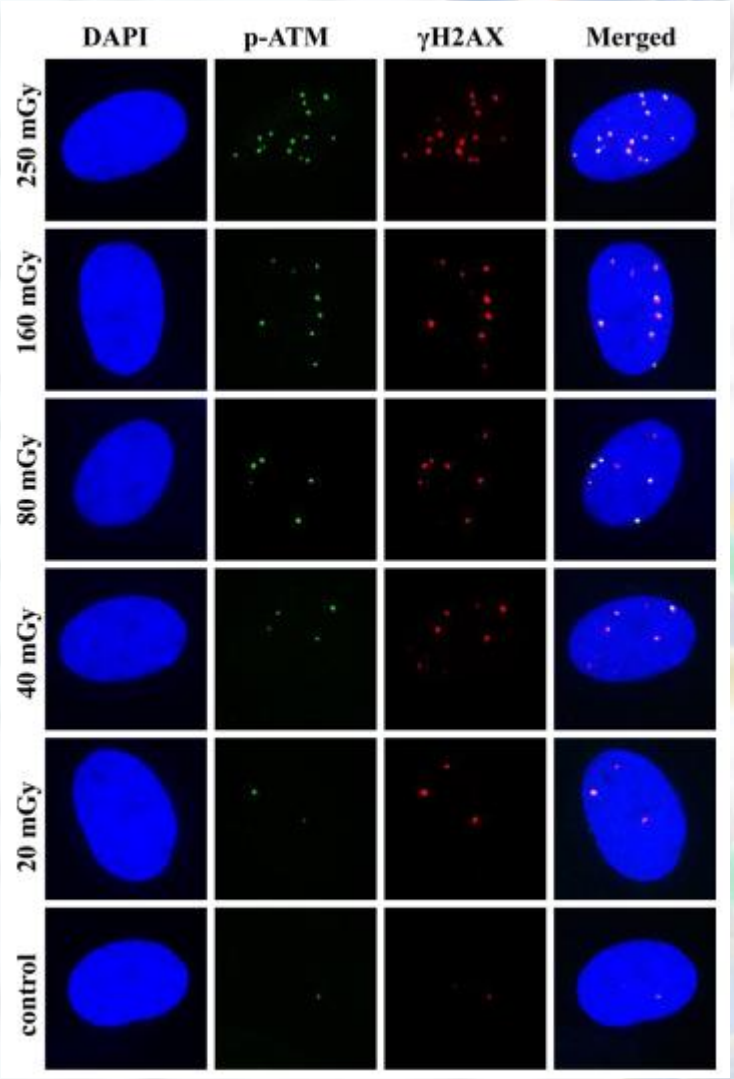
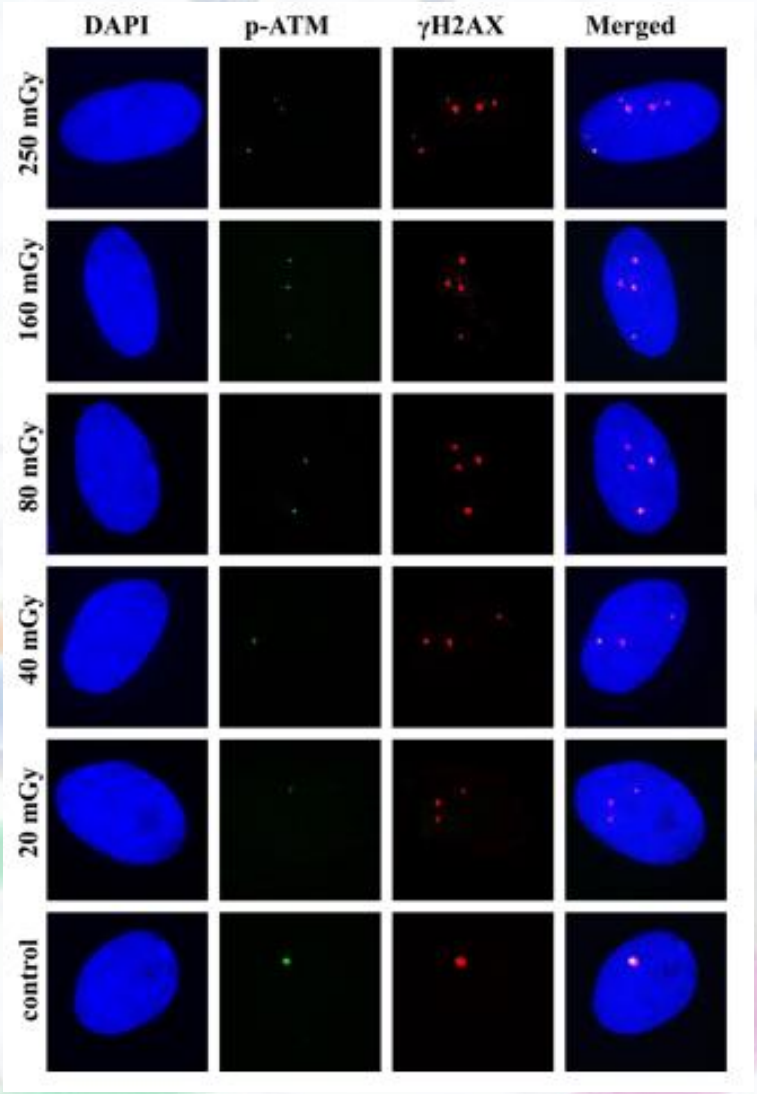


Figure 3: Kinetics of γH2AX, but not pATM, foci induced in MSCs is dose-dependent. Cells were exposed to 250 mGy **(A)** 160 mGy **(B)** 80 mGy **(C)** 40 mGy **(D)** 20 mGy **(E)** or left untreated **(F)** and fixed at various indicated time-points after irradiation up to 240 min. Number of γH2AX and pATM foci were quantified, as well as their co-localization and mean values from three independent experiments ± SD were plotted.

30 min post-irradiation



240 min post-irradiation



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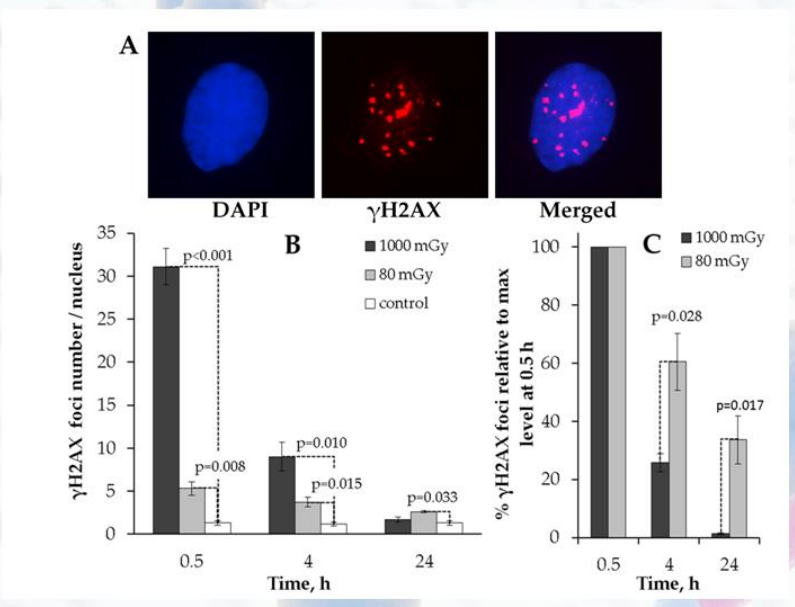
Research Paper

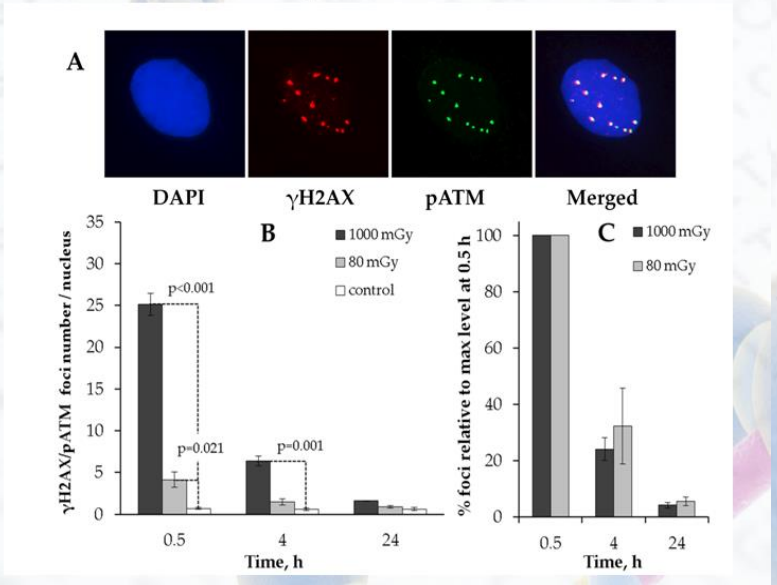
Residual γH2AX foci induced by low dose x-ray radiation in bone marrow mesenchymal stem cells do not cause accelerated senescence in the progeny of irradiated cells

Margarita Pustovalova[1,2], Tatiana A. Astrelina[1], Anna Grekhova[1,2,3], Natalia Vorobyeva[1,2], Anastasia Tsvetkova[1,4], Taisia Blokhina[1,2], Victoria Nikitina[1], Yulia Suchkova[1], Daria Usupzhanova[1], Vitalyi Brunchukov[1], Irina Kobzeva[1], Tatiana Karaseva[1], Ivan V. Ozerov[1,5], Aleksandr Samoylov[1], Andrey Bushmanov[1], Sergey Leonov[4,6], Evgeny Izumchenko[7], Alex Zhavoronkov[5], Dmitry Klokov[8,9], Andreyan N. Osipov[1,2,4,5]

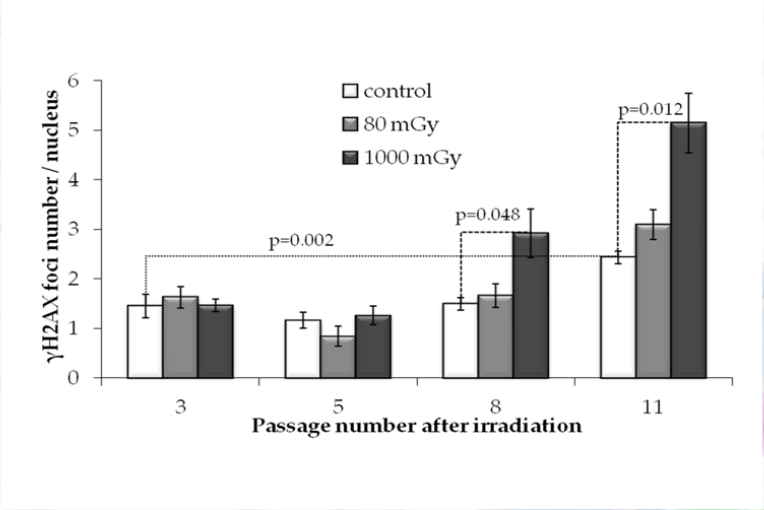
Low dose X-rays induces persistent γH2AX foci



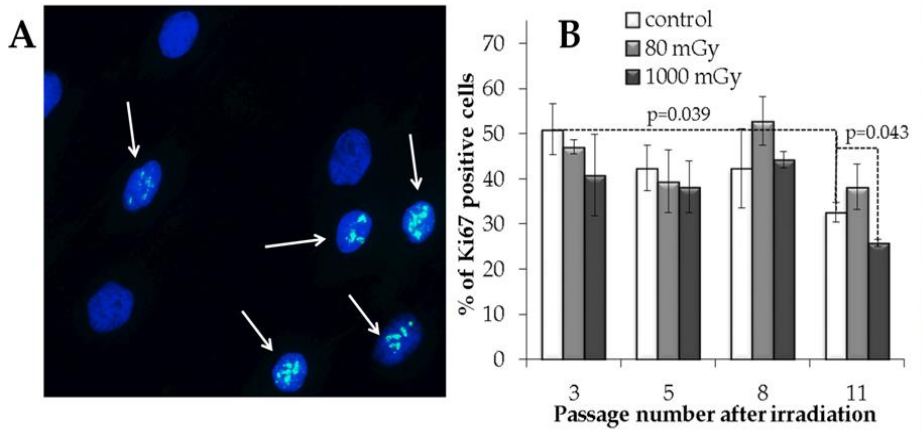
Residual γH2AX foci induced by low-dose X-rays are not co-localized with pATM foci



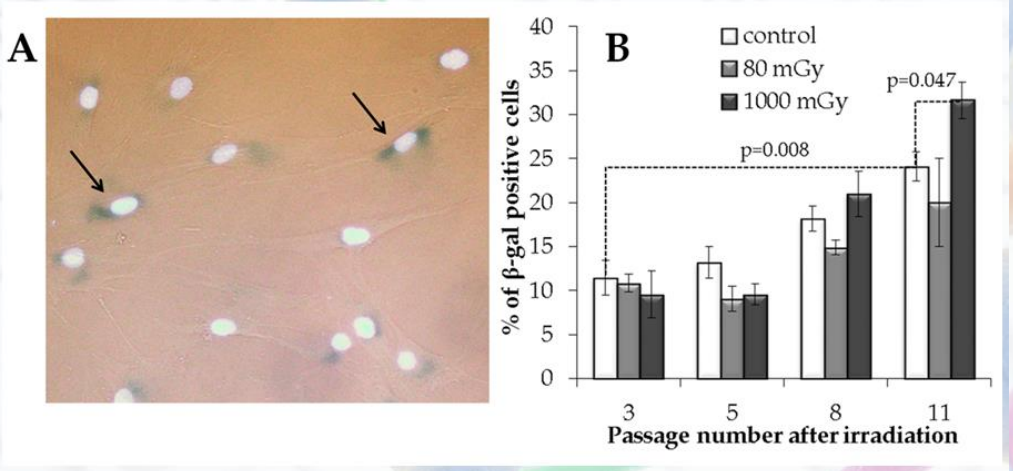
Low-dose X-rays do not cause an increase in the γH2AX foci number in the progeny of irradiated cells



Low-dose X-rays do not decrease proliferation activity in the progeny of irradiated cells

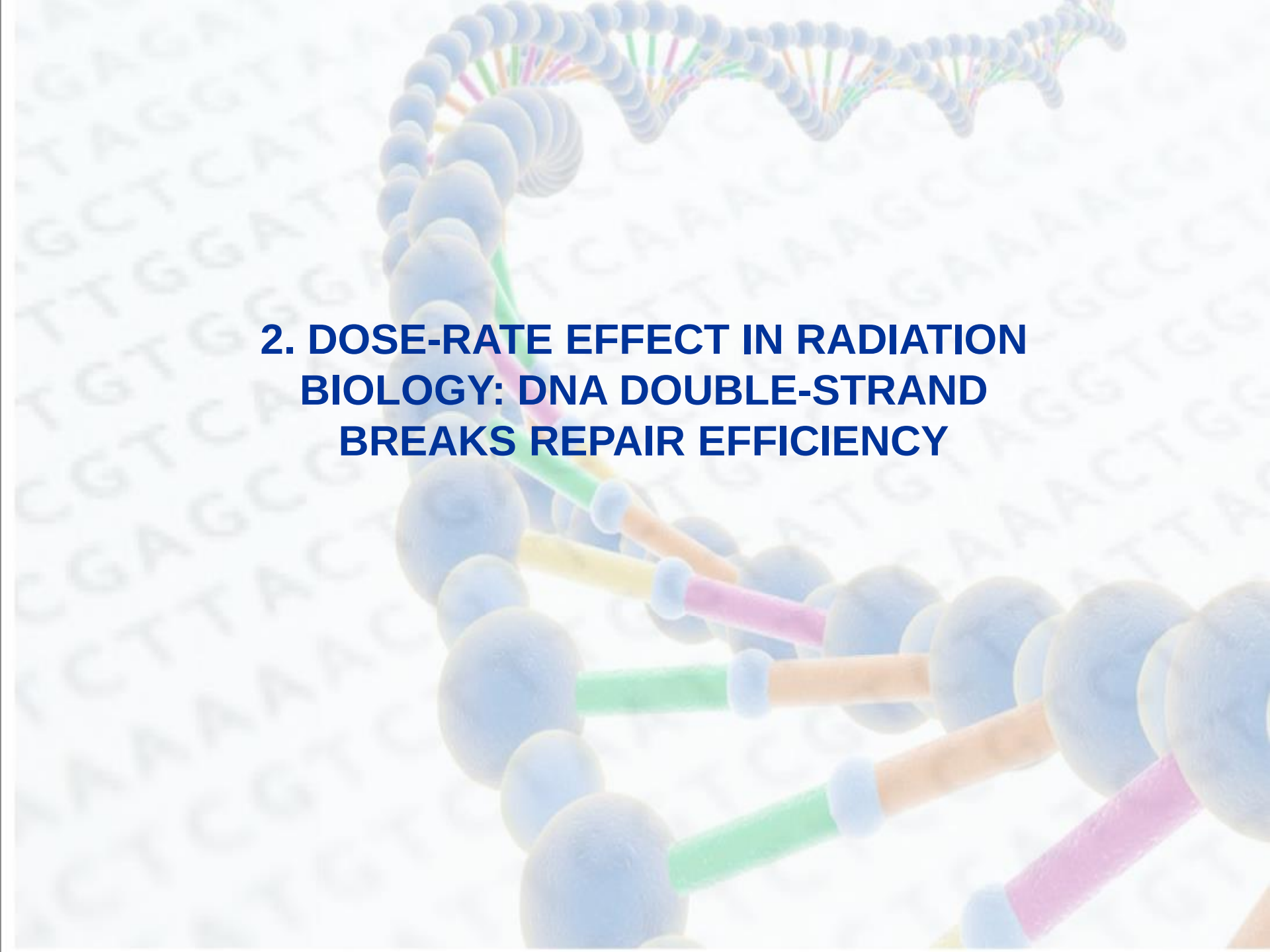


Low-dose X-rays do not accelerate senescence in the progeny of irradiated cells



Our results indicate that although excess γH2AX foci were present at 24 h post-irradiation with 80 mGy, a finding that is commonly interpreted as presenting mutagenic potential and subsequently health risk, the progeny of the irradiated cells did not display health abnormalities, such as increased senescence, suppressed proliferation and high γH2AX foci rates. We conclude that accurate interpretation of γH2AX foci measurements may require additional assays, such as quantification of pATM foci, proliferation and senescence, over extended periods of time. Therefore, care must be taken when using γH2AX in biodosimetry or in accessing individual rediosensitivity since time of sampling after irradiation may yield inconsistent results.

2. DOSE-RATE EFFECT IN RADIATION BIOLOGY: DNA DOUBLE-STRAND BREAKS REPAIR EFFICIENCY



Dose-rate effect

Dose rate determines biological impact

- reduction in dose rate causes reduced cell killing, due to repair of SLD
- reduction in dose rate generally reduces survival-curve slope (D0 increases)
- inverse dose-rate effect occurs in some cell lines at 'optimal' dose rate due to accumulation of cells in G2

Broad shoulder to survival curve

Dose-Rate Effect in CHO Cells

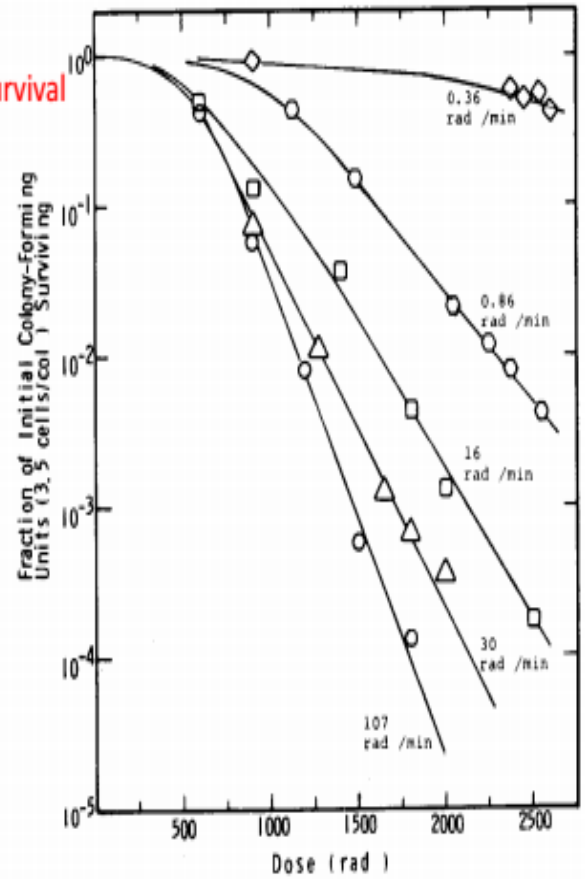


Figure 5.10. Dose–response curves for Chinese hamster cells (CHL-F line) grown *in vitro* and exposed to cobalt-60 γ-rays at various dose rates. At high doses a substantial dose-rate effect is evident even among 1.07, 0.3, and 0.16 Gy/min (107, 30, and 16 rad/min). The decrease in cell killing becomes even more dramatic as the dose rate is reduced further. (From Bedford JS, Mitchell JB: Dose rate effects in synchronous mammalian cells in culture. Radiat Res 54:316–327, 1973, with permission.)

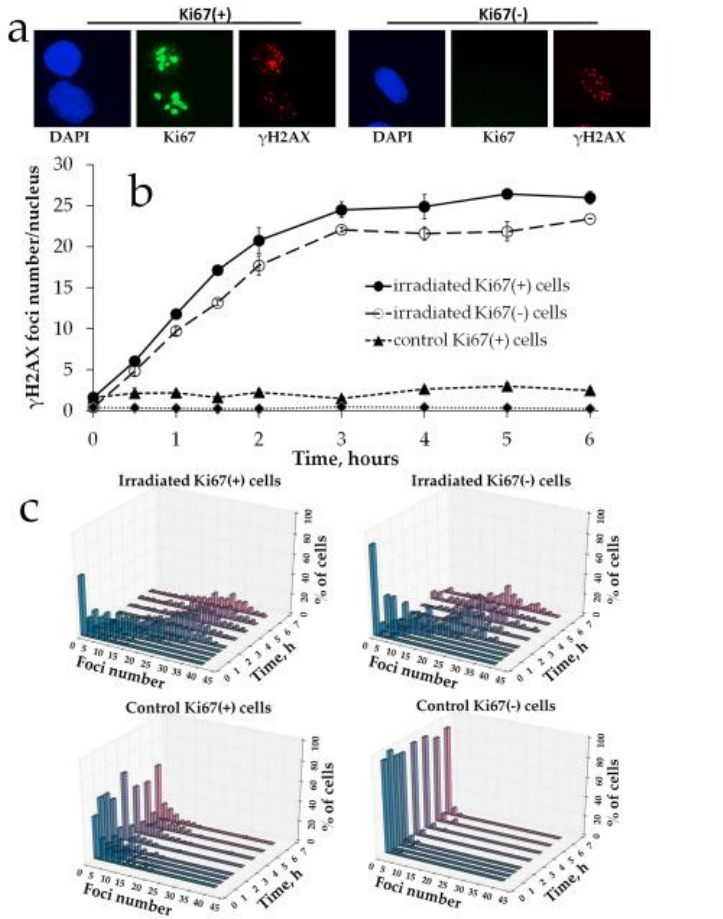


Figure 3: γH2AX foci formation in proliferating vs. resting MSCs exposed to prolonged X-ray irradiation. (a) Representative microphotographs of immunofluorescently stained irradiated MSCs showing Ki67 (green) and γH2AX foci (red). DAPI counterstaining is shown in blue. **(b)** Quantification of γH2AX in Ki67+ vs Ki67- MSCs exposed to prolonged (270 mGy/h) X-ray irradiation. Mean foci numbers derived from at least three independent experiments are shown. Error bars show SE. **(c)** Histograms showing percent of cells with a certain number of γH2AX foci.

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 38), pp: 64317-64329

Research Paper

γH2AX, 53BP1 and Rad51 protein foci changes in mesenchymal stem cells during prolonged X-ray irradiation

Anastasia Tsvetkova[1], Ivan V. Ozerov[2,3], Margarita Pustovalova[2,4], Anna Grekhova[2,4,5], Petr Eremin[6], Natalia Vorobyeva[2,3], Ilya Eremin[6], Andrey Pulin[6], Vadim Zorin[6,7], Pavel Kopnin[8], Sergey Leonov[9], Alex Zhavoronkov[3], Dmitry Klokov[10] and Andreyan N. Osipov[2,3,4,9]

The experimental results indicate that kinetics of DNA double-strand break formation upon continuous exposure to X-ray radiation at a dose-rate of 4.5 mGy/min monitored in cells using γH2AX/53BP1 foci consists of two components: i) linear accumulation with time (dose) of exposure, and ii) plateau. The plateau reflects the result of two simultaneously occurring processes in continuously exposed cells: DSBs induction and their repair.

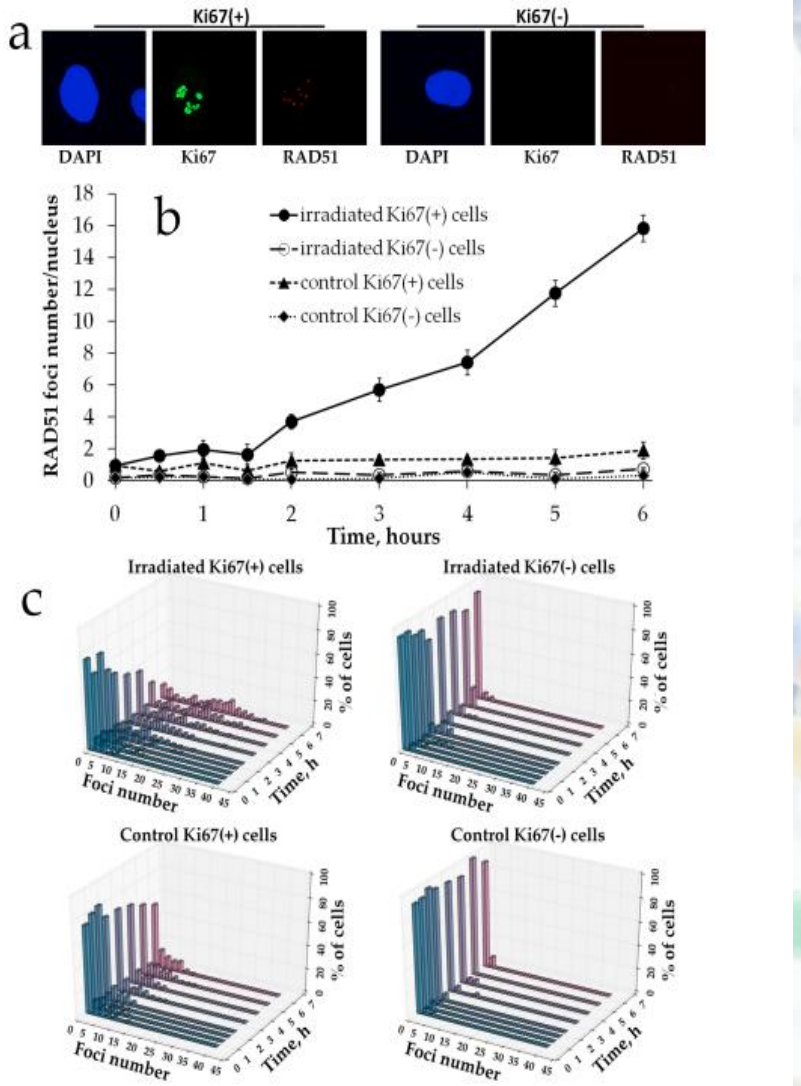


Figure 4: Rad51 foci formation in proliferating vs. resting MSCs exposed to prolonged X-ray irradiation. (a) Representative microphotographs of immunofluorescently stained irradiated MSCs showing Ki67 (green) and Rad51 foci (red). DAPI counterstaining is shown in blue. (b) Quantification of Rad51 in Ki67+ vs Ki67- MSCs exposed to prolonged (270 mGy/h) X-ray irradiation. Mean foci numbers derived from at least three independent experiments are shown. Error bars show SE. (c) Histograms showing percent of cells with a certain number of Rad51 foci.

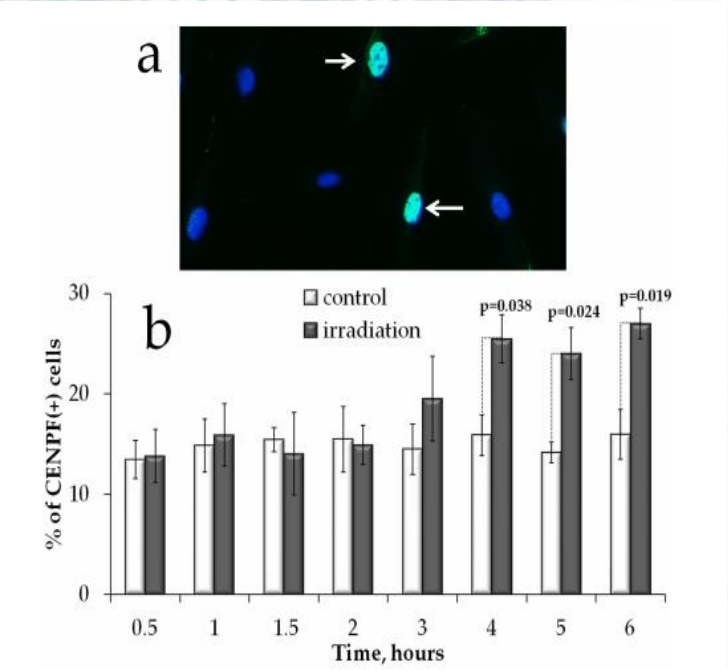
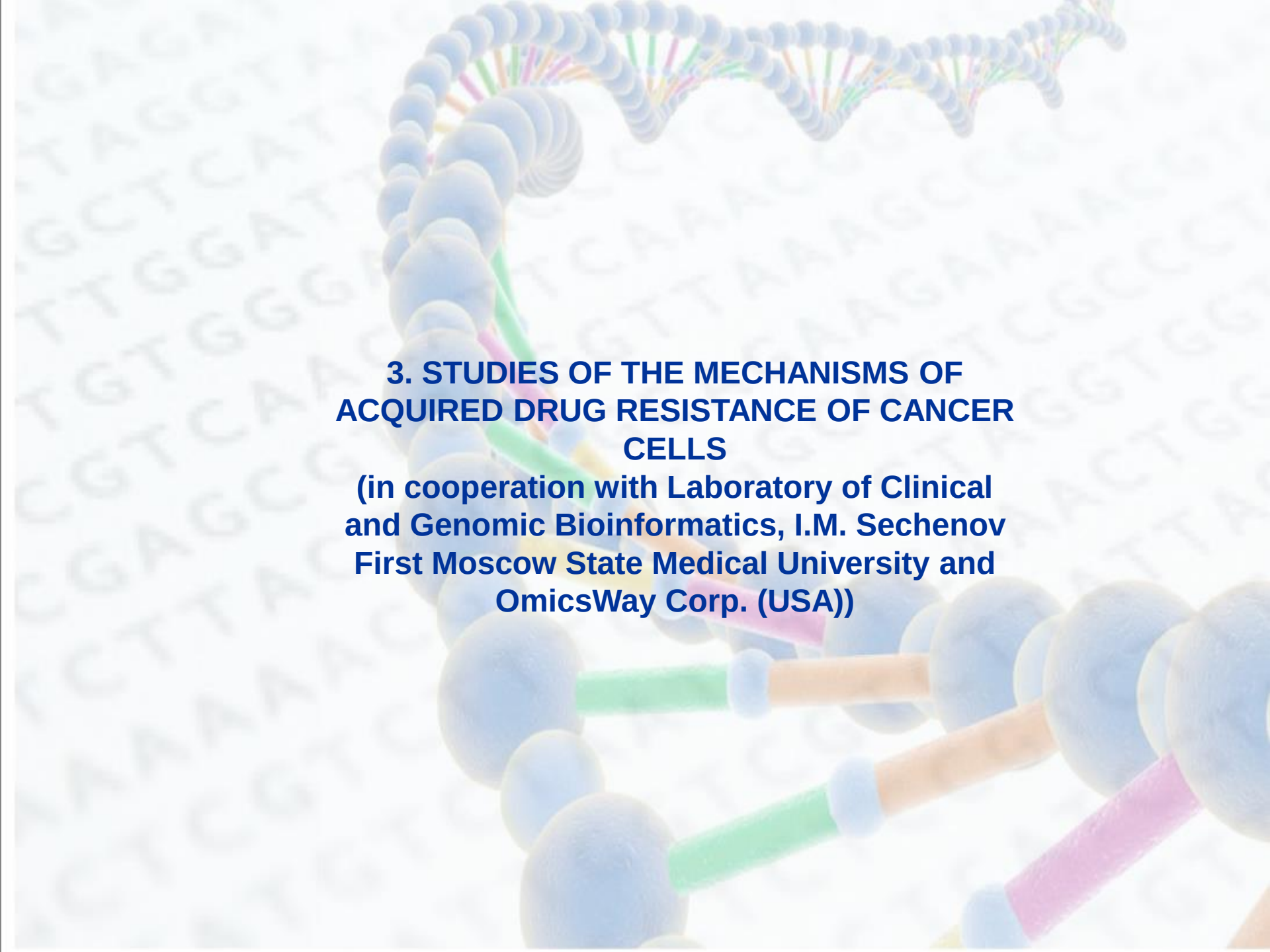


Figure 6: S/G2 cell cycle phases changes in MSCs exposed to prolonged irradiation. (a) Representative microphotographs of immunofluorescently stained irradiated MSCs showing CENPF (green) DAPI counterstaining (blue). (b) Quantification of CENPF+ cells in cultures exposed to prolonged (270 mGy/h) X-ray irradiation. Mean values derived from at least three independent experiments are shown. Error bars show SE. p-values of statistically significant differences are shown.

Our data suggest that prolonged exposure of cells to ionizing radiation leads to accumulation of cells in S/G2 phases of the cell cycle and associated activation of homologous recombination.

Also, proliferation status may significantly affect the biological outcome, since homologous repair is not activated in resting cells.

Our finding show principal differences in the contribution of non-homologous end-joining and homologous recombination to the repair of DNA double-strand breaks in mammalian cells irradiated at different dose-rates. During continuous irradiation, accumulation of cells in S/G2 phases and associated activation of homologous recombination DNA DSB repair pathway are observed. The observed activation of the error-free DNA DSB repair pathway suggests compensatory adaptive mechanisms that may help alleviate long-term biological consequences and could potentially be utilized both in radiation protection and medical practices.



3. STUDIES OF THE MECHANISMS OF ACQUIRED DRUG RESISTANCE OF CANCER CELLS

(in cooperation with Laboratory of Clinical and Genomic Bioinformatics, I.M. Sechenov First Moscow State Medical University and OmicsWay Corp. (USA))

www.impactjournals.com/oncotarget/ Oncotarget, 2018, Vol. 9, (No. 4), pp: 5111-5124

Research Paper

Acquired resistance to tyrosine kinase inhibitors may be linked with the decreased sensitivity to X-ray irradiation

Maxim Sorokin[1,2,3], Roman Kholodenko[3], Anna Grekhova[4], Maria Suntsova[1,5], Margarita Pustovalova[4], Natalia Vorobyeva[1,4], Irina Kholodenko[6], Galina Malakhova[2], Andrew Garazha[1,7], Artem Nedoluzhko[2], Raif Vasilov[2], Elena Poddubskaya[8], Olga Kovalchuk[9], Leila Adamyan[10], Vladimir Prassolov[5], Daria Allina[11], Denis Kuzmin[12], Kirill Ignatev[13], Andreyan Osipov[1,4] and Anton Buzdin[2,3,5,7]

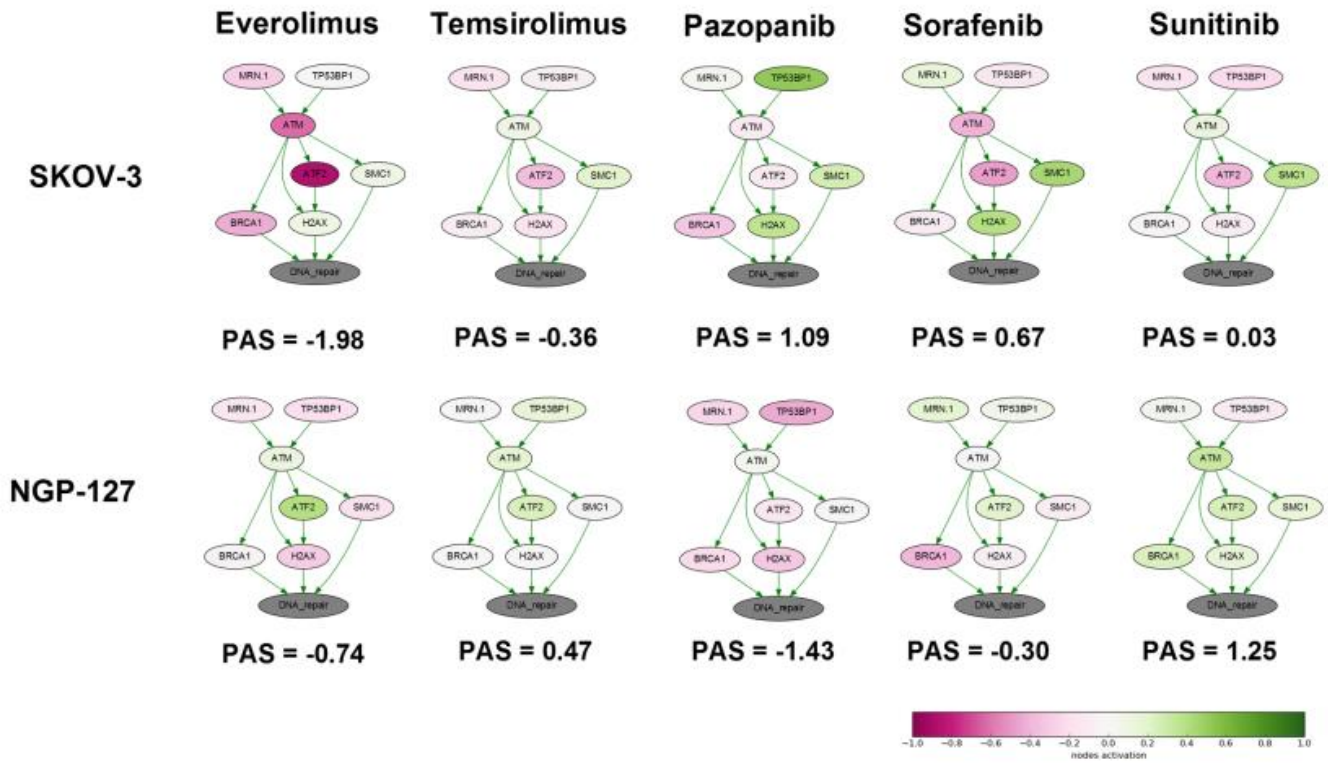


Figure 1: Schematic representation of alterations in "ATM Pathway (DNA repair)" molecular pathway after 4 weeks of incubation with target drugs. The pathway is shown as an interacting network, where green arrows indicate activation, red arrows – inhibition. Pathway Activation Strength score is shown for each sample. Color depth corresponds to the logarithms of the case-to-normal (CNR) expression rate for each node, where "normal" is a geometric average between control samples. Exact CNR values are provided in Supplementary Table 2.

For the SKOV-3 (ovarian carcinoma), but not NGP-127 (neuroblastoma) cells, for the the clinically relevant tyrosine kinase inhibitors (TKIs) Sorafenib, Pazopanib and Sunitinib, we noticed statistically significant increase in capacity to repair radiation-induced DNA double strand breaks compared to naïve control cells not previously treated with TKIs. These peculiarities were linked with the increased activation of ATM DNA repair pathway in the TKI-treated SKOV-3, but not NGP-127 cells. Our results provide a new cell culture model for studying anti-cancer therapy efficiency and evidence that there may be a tissue-specific radioresistance emerging as a side effect of treatment with TKIs.

4. STUDIES OF THE MECHANISMS OF RADIORESISTANCE OF CANCER CELLS
(in cooperation with School of Biological and Medical Physics, Moscow Institute of Physics and Technology)

International Journal of *Molecular Sciences*

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Article

The p53–53BP1-Related Survival of A549 and H1299 Human Lung Cancer Cells after Multifractionated Radiotherapy Demonstrated Different Response to Additional Acute X-ray Exposure

Margarita Pustovalova [1,2,*], Lina Alhaddad [1], Nadezhda Smetanina [1,2], Anna Chigasova [1,3], Taisia Blokhina [1,2,4], Roman Chuprov-Netochin [1], Andreyan N. Osipov [1,2,4,*] and Sergey Leonov [1,5,*]

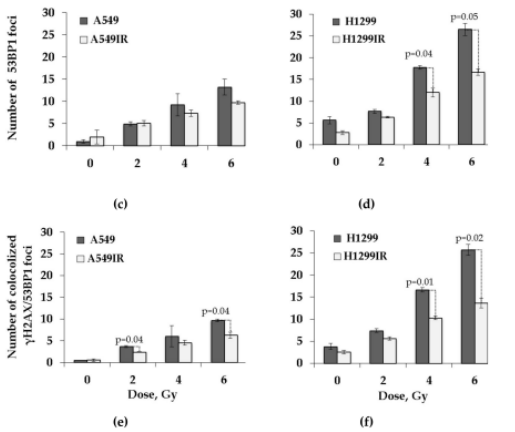


Figure 8. Changes in the number of residual γH2AX, 53BP1 foci, and their co-localization was analyzed in A549 and A549IR cells and H1299 and H1299IR cells 24 h after exposure to different single doses of X-rays. The number of residual γH2AX foci increased post-IR at extra single doses in A549 and A549IR cells (**a**) and H1299 and H1299IR cells (**b**). Changes in the number of residual 53BP1 in A549 and A549IR cells (**c**) and H1299 and H1299IR cells (**d**). Changes in the number of co-localized γH2AX/53BP1 in A549 and A549IR cells (**e**) and H1299 and H1299IR cells (**f**). Data are means ± SEM of more than three independent experiments.

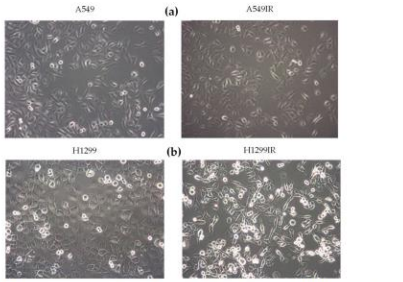


Figure 1. Phase contrast microscopic images of parental and irradiation-surviving A549 (**a**) and H1299 (**b**) cells. Objective: 20x.

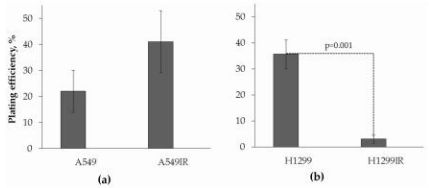


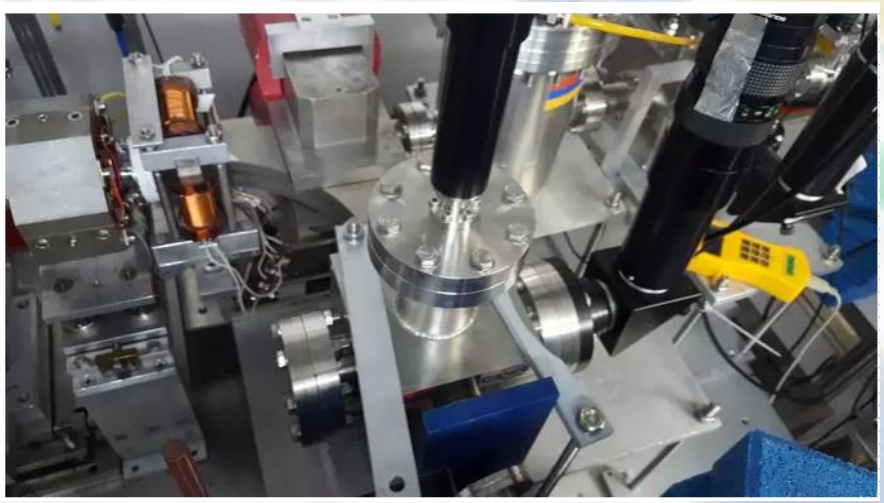
Figure 2. Plating efficiency of parental and irradiation-surviving A549 (**a**) and H1299 (**b**) cells. Colonies of the small size were counted under light microscope. Colonies ≥ 50 cells were counted. Data are means ± SEM of more than three independent experiments.

Our study provides strong evidence that different DNA repair mechanisms are activated by multifraction radiotherapy (MFR), as well as single-dose IR, and that the enhanced cellular survival after MFR is reliant on both p53 and 53BP1 signaling along with non-homologous end-joining (NHEJ). The results are of clinical significance as they can guide the choice of the most effective IR regimen by analyzing the expression status of the p53–53BP1 pathway in tumors and thereby maximize therapeutic benefits for the patients while minimizing collateral damage to normal tissue.

5. DNA DOUBLE-STRAND BREAK REPAIR EFFICIENCY IN CANCER CELLS EXPOSED TO LASER-DRIVEN ULTRASHORT ELECTRON BEAMS

(in cooperation with Semenov Institute of Chemical Physics (Russian Academy of Sciences), CANDLE Synchrotron Research Institute (Armenia), Yerevan State University and Institute of Molecular Biology (Armenia))

Over the past few years, a new direction in radiation biology related to the study of the mechanisms of ultrashort pulsed radiation (femto- and picoseconds) effects formation has begun to develop. In case of such short pulse duration only ionization and the formation of free radicals may occur, while all chemical processes begin later. At the same time, during the pulse, the peak dose-rate values up to GGy/sec may be achieved. The radiobiological effects of irradiation with this kind of characteristics (ultrashort duration and ultrahigh peak-dose) are poorly studied.



International Journal of
Molecular Sciences

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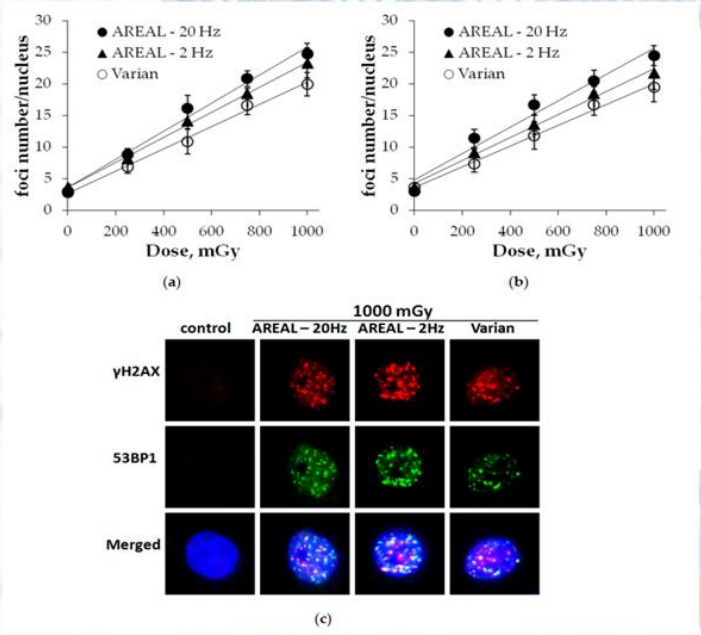
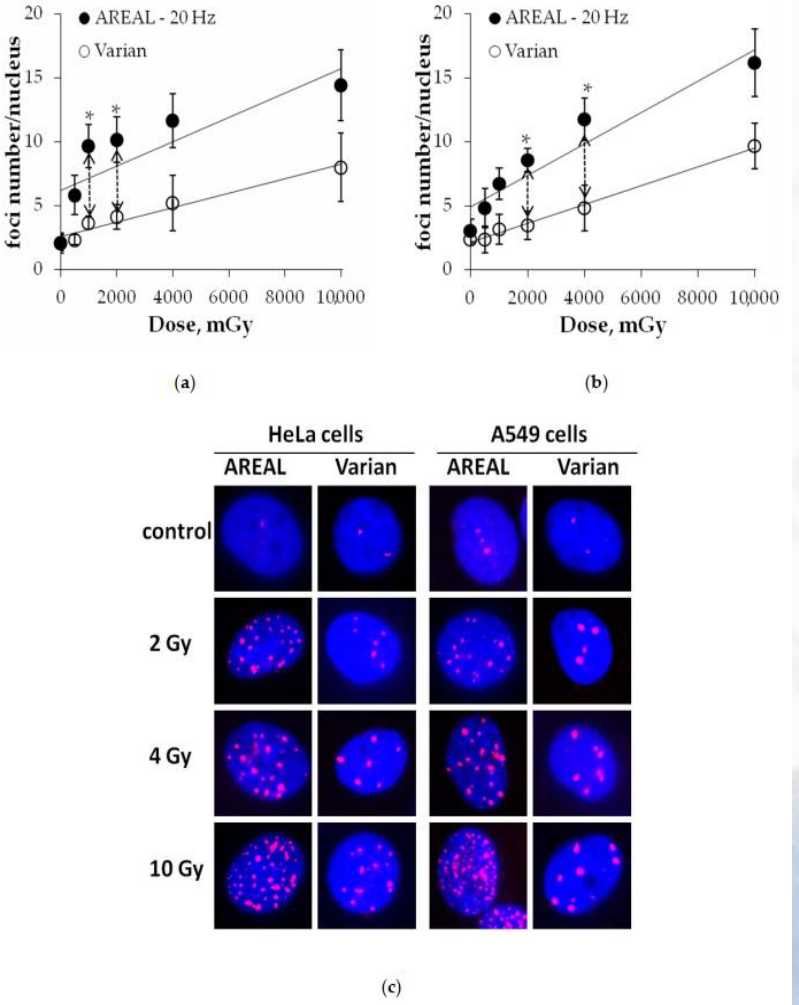
Communication

Low Repair Capacity of DNA Double-Strand Breaks Induced by Laser-Driven Ultrashort Electron Beams in Cancer Cells

Nelly Babayan [1,2], Natalia Vorobyeva [3,4], Bagrat Grigoryan [5], Anna Grekhova [4,6], Margarita Pustovalova [7], Sofya Rodneva [3], Yuriy Fedotov [3], Gohar Tsakanova [1,5], Rouben Aroutiounian [2] and Andreyan Osipov [3,4,7,*]



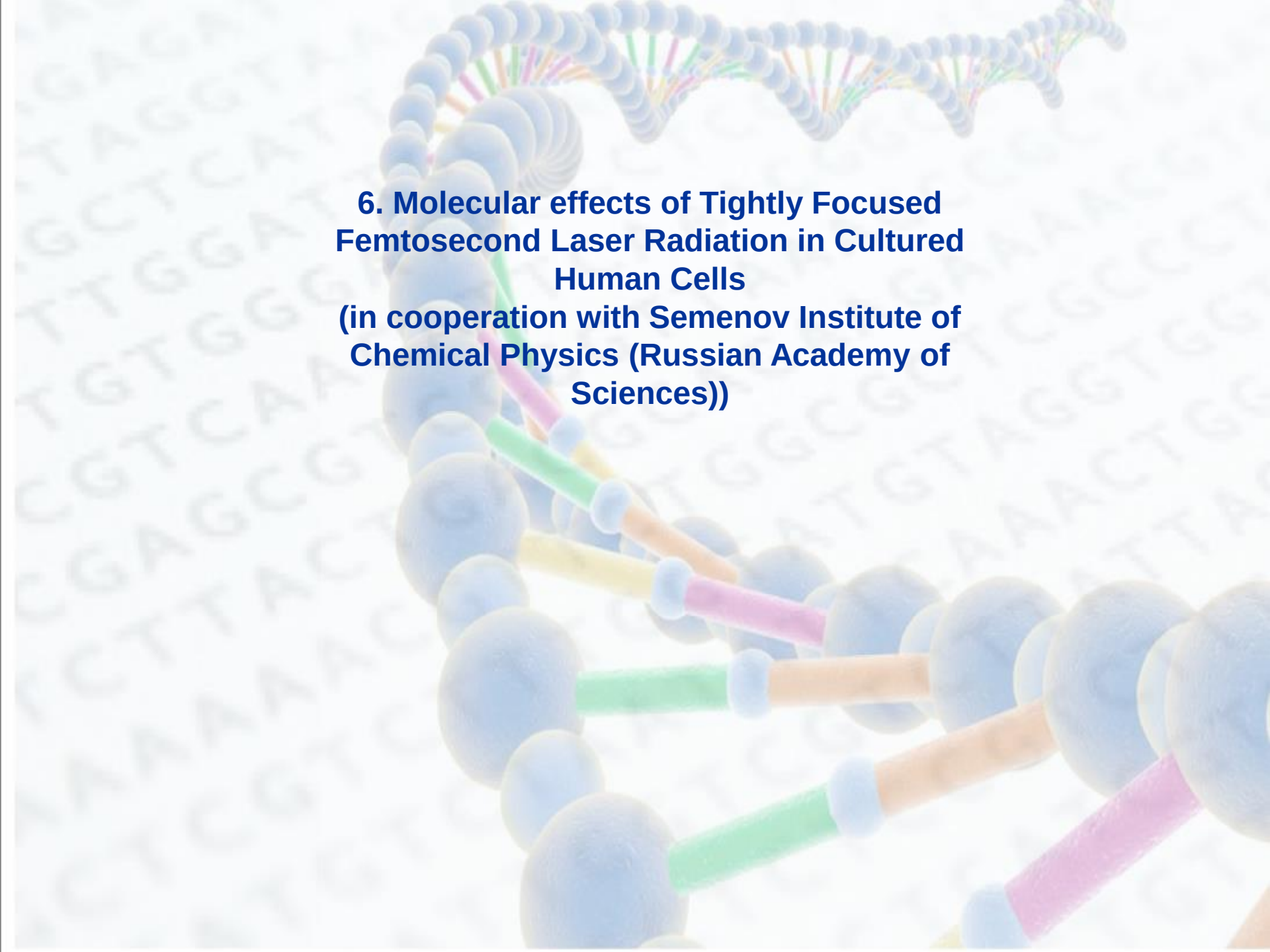
Dose-dependent changes in γH2AX (a) and 53BP1 (b) foci numbers in HeLa cells at 1h post-irradiation by AREAL and Variant accelerators.



Dose-dependent changes in γH2AX residual foci numbers in HeLa (a) and A549 (b) cells at 24 h post-irradiation by AREAL and Varian accelerators.

In general, the obtained data indicated slower DSB repair rate induced by ultrashort pulsed irradiation, compared to the ones induced by quasi-continuous irradiation. The pulse duration of ultrashort irradiation is only 0.4×10^{-12} s, however, a huge peak dose-rate of 1.6×10^{10} Gy/s per pulse is achieved during the pulse. Apparently, it increases the possibility of complex difficulty repairable DSBs formation. Further detailed studies of the physicochemical mechanisms of biological effects induced by sub-picosecond pulse irradiation are needed.

6. Molecular effects of Tightly Focused Femtosecond Laser Radiation in Cultured Human Cells (in cooperation with Semenov Institute of Chemical Physics (Russian Academy of Sciences))



molecules
MDPI

Communication

Immunocytochemical Localization of XRCC1 and γH2AX Foci Induced by Tightly Focused Femtosecond Laser Radiation in Cultured Human Cells

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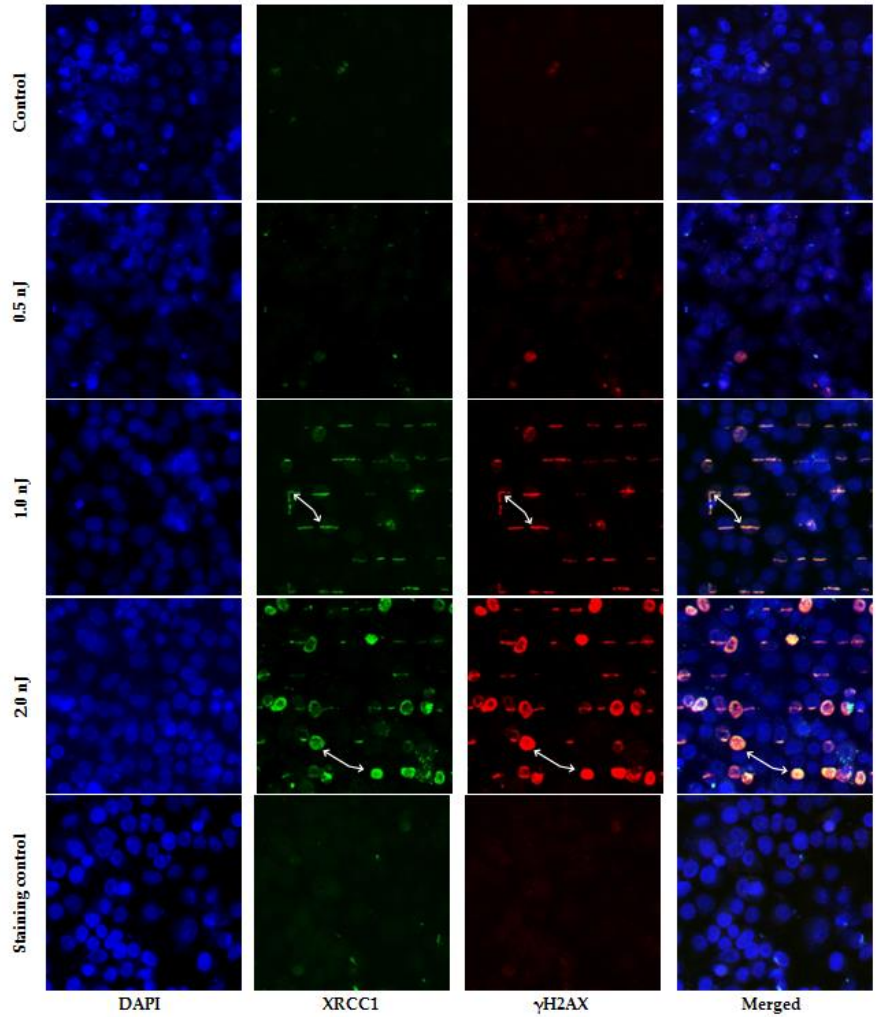

Figure 1. Microscopy image of immunocytochemically stained cell nuclei 30 min after exposure to femtosecond laser radiation with a pulse energy of 0.5 nJ (power density 1×10^{11} W cm^{-2}), 1.0 nJ (power density 2×10^{11} W cm^{-2}), and 2.0 nJ (power density 4×10^{11} W cm^{-2}). Arrows show foci tracks (1.0 nJ) or pan-stained nuclei (2.0 nJ). Staining control cells irradiated with a pulse energy of 2.0 nJ and stained with only secondary antibodies.

Femtosecond laser radiation in the near infrared range (800–1100 nm) is widely used in biological research, including as an ultra-precise scalpel for nanosurgical treatment. The physicochemical basis of this application is based on the principles of nonlinear absorption of laser pulses with a high peak power and the subsequent formation of low density plasma in the absorption region of a femtosecond laser pulse. Our results showed that femtosecond laser pulses of 10^{11} W·cm^{-2} peak power density led to the formation of linear tracks consisting both of XRCC1 and γH2AX protein foci localized in the places where the laser beam passed through the cell nuclei. A further increase in the pulse power density to 4×10^{11} W·cm^{-2} led to the appearance of nuclei with total immunocytochemical staining for XRCC1 and γH2AX on the path of the laser beam. Thus, femtosecond laser radiation can be considered as a tool for local ionization of biological material, and this ionization will lead to similar effects obtained using ionizing radiation.

7. EFFECTS OF COMBINED EXPOSURE TO MODELED RADIATION AND GRAVITATION FACTORS OF THE INTERPLANETARY FLIGHT (in cooperation with Institute of Biomedical Problems of the Russian Academy of Sciences and Joint Institute for Nuclear Research)

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Effects of combined exposure to modeled radiation and gravitation factors of the interplanetary flight: Monkeys' cognitive functions and the content of monoamines and their metabolites; cytogenetic changes in peripheral blood lymphocytes

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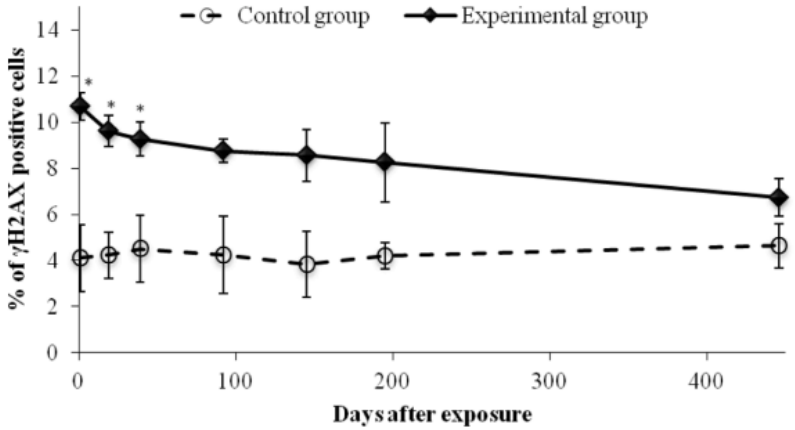

Fig. 7. The dynamics of the proportion of γH2AX-positive peripheral blood lymphocytes in rhesus macaques after the end of a complex exposure, which modeled space flight conditions (hypokinesia + prolonged γ-irradiation + accelerated ^{12}C ion exposure). The data are shown as the arithmetic mean ± standard error; statistical significance was tested using the Student *t*-test. * - p value < 0.05. The dashed line shows the control values; the solid line shows the exp animals' data.

In our research, 24 h after the end of a complex exposure, which modeled space flight conditions (hypokinesia + prolonged γ-ray irradiation + accelerated ^{12}C ion exposure), flow cytometry analysis of γH2AX-positive cells showed a statistically significant (p = 0.014) 2.6-fold increase in the percent of γH2AX-positive peripheral blood lymphocytes compared with the control level (Fig. 7). Contrary to our expectations, 19 days after the end of the exposures, only a minor decrease was observed in the percent of γH2AX-positive cells (down to 2.3 times the control level, p = 0.011). Moreover, even after 42 days, the percent of γH2AX-positive cells remained increased (2.1 times the control level, p = 0.042). Then, the γH2AX-positive cell percent slowly decreased; by the 92nd day, its excess over the control level was not statistically significant (p = 0.063). However, up to 446 days, a clear trend for maintaining an increased γH2AX-positive cell yield was observed.


Thank you for your time attention!!!