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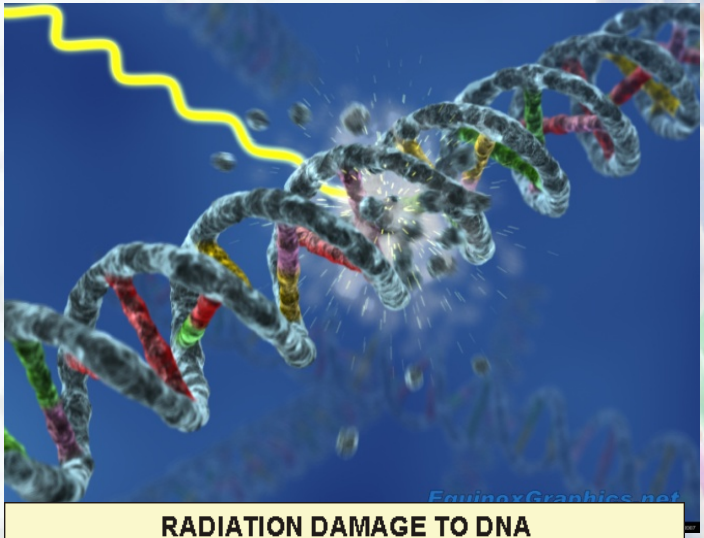
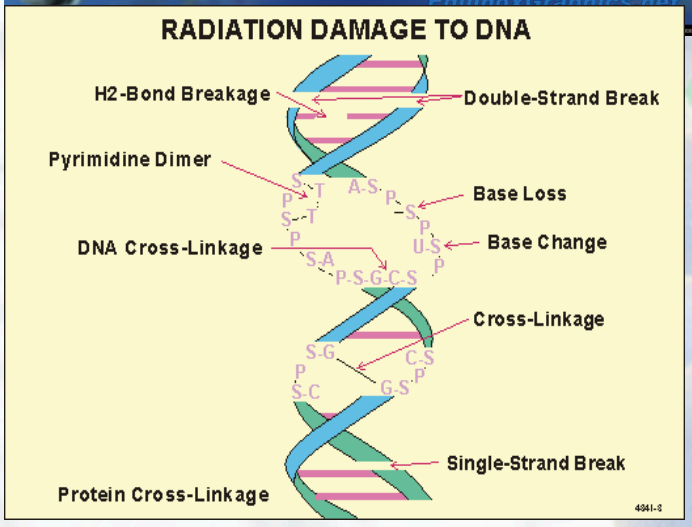
Immunocytochemical detection of radiation-induced DNA damage

Andreyan Osipov

Ultrafast Beams and Applications,
02-05 July 2019, CANDLE, Armenia

Introduction





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.

DNA double-strand breaks (DSBs) are the most crucial DNA lesions introduced by the exposure of cells to ionizing radiation (IR).

Approximately 8 out of 10 IR-induced DSBs are repaired by the relatively fast but error-prone non-homologous end-joining (NHEJ) pathway. This can lead to formation of the deleterious microdeletions and chromosome aberrations in the cell. Inaccurately repaired DSBs may lead to cell death, mutagenesis and carcinogenesis.

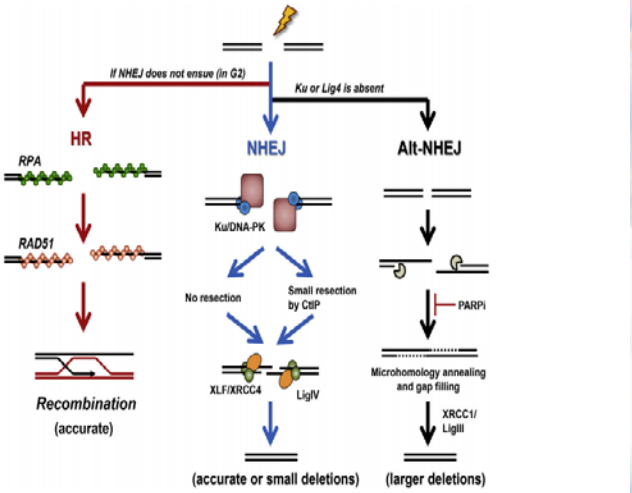


Fig 1. Pathways of double-strand break (DSB) rejoining and their hierarchy. Non-homologous end-joining (NHEJ) is the first choice DSB repair pathway in mammalian cells. However, pathways exploiting resection can be used if rapid repair by NHEJ does not ensue. Homologous recombination can be used in late 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

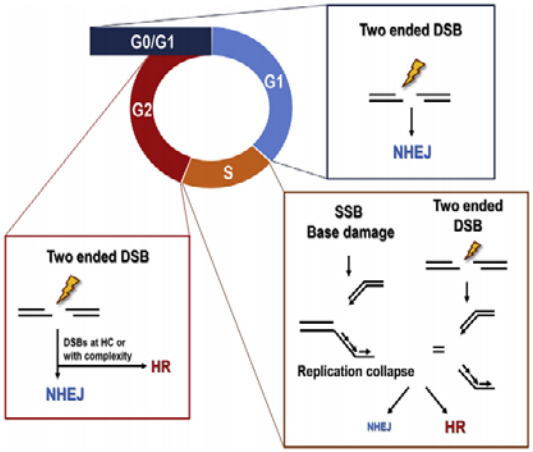
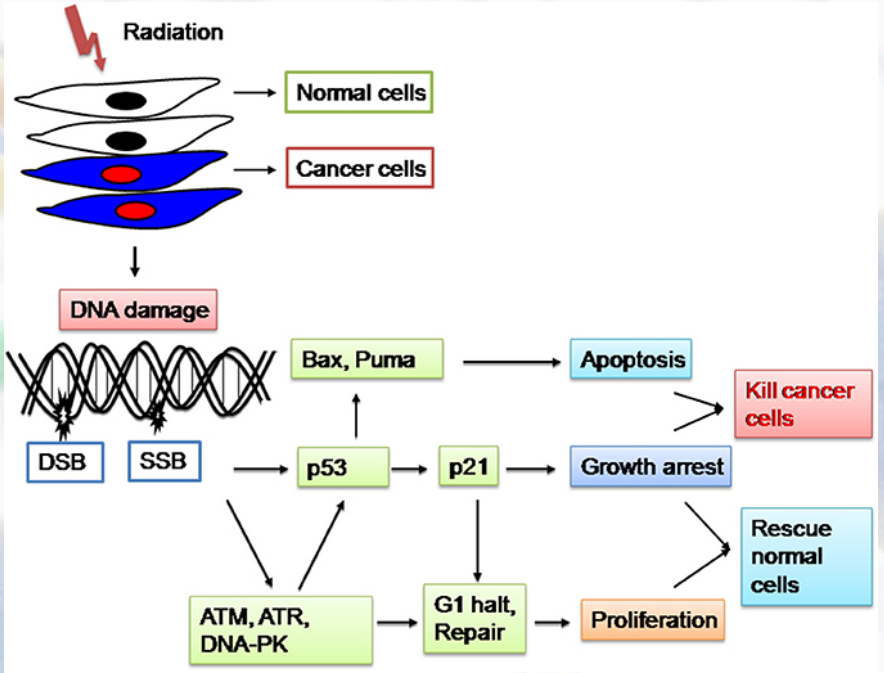
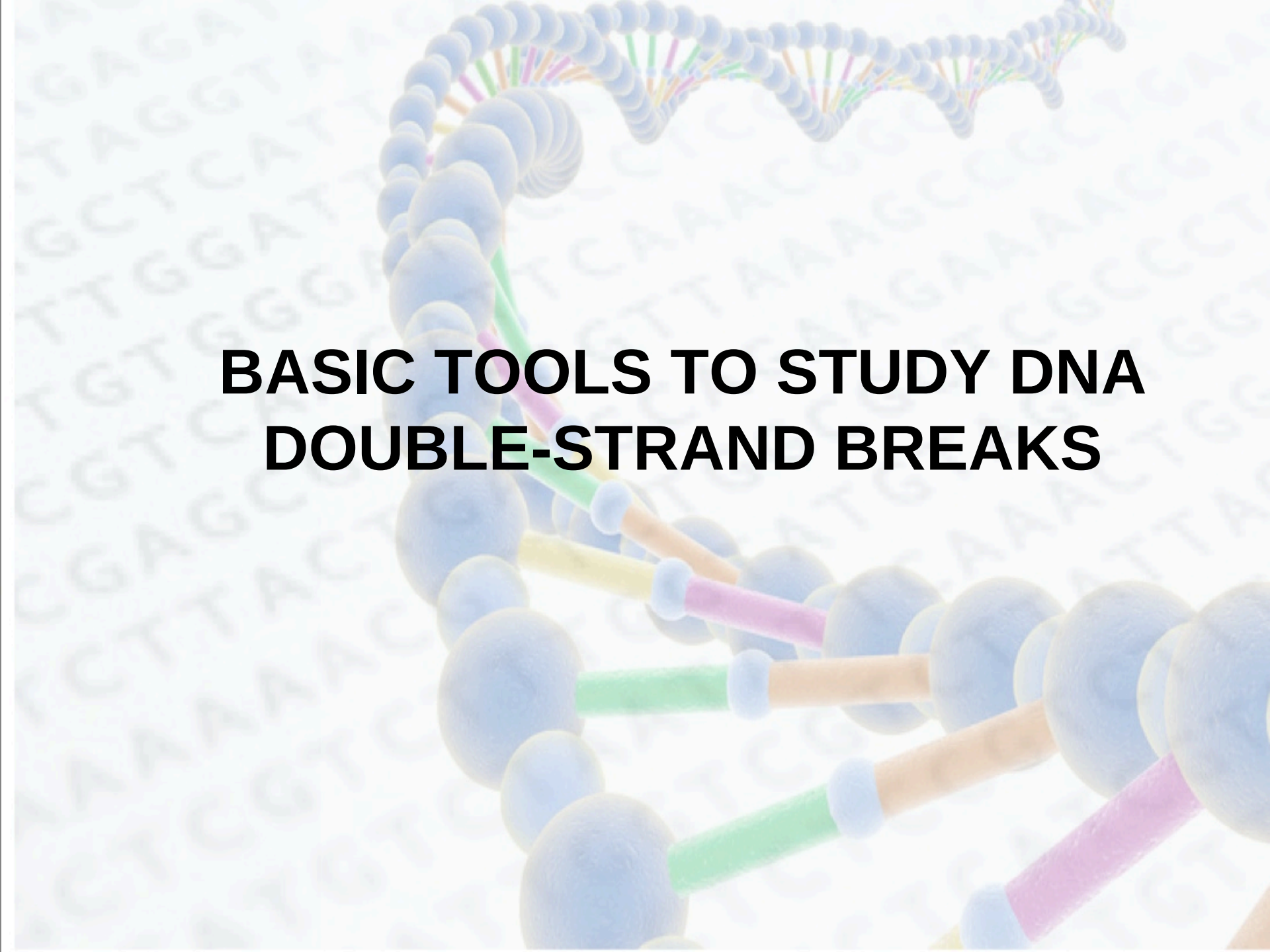


Fig 2. Cell cycle regulation of non-homologous end-joining (NHEJ) and homologous recombination. NHEJ can function in all cell cycle phases. Homologous recombination has a major role promoting recovery from replication fork stalling in S phase but can also function to repair radiation-induced two-ended 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.



MOLECULAR BIOSCIENCES
REVIEW ARTICLE
Biological response of cancer cells to radiation treatment

BASIC TOOLS TO STUDY DNA DOUBLE-STRAND BREAKS



Pulsed-field gel electrophoresis

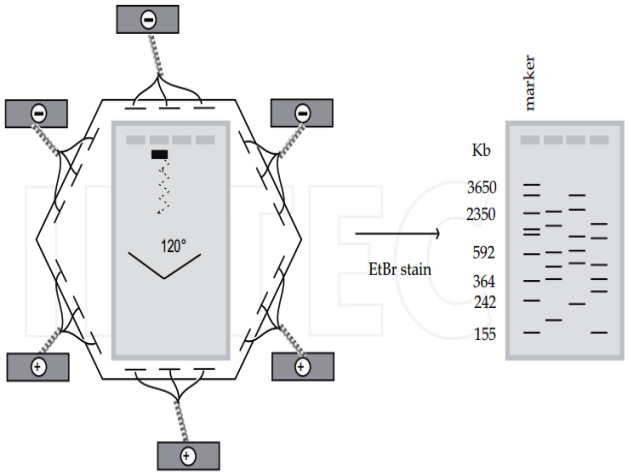


Fig. 1. Schematic diagram of PFGE instrumentation. Contoured clamped homogeneous electric field (CHEF) systems use a hexagonal gel box that alters the angle of the fields relative to the agarose gel. After running the gel by PFGE, DNA fragments are visualized by staining with ethidium bromide.

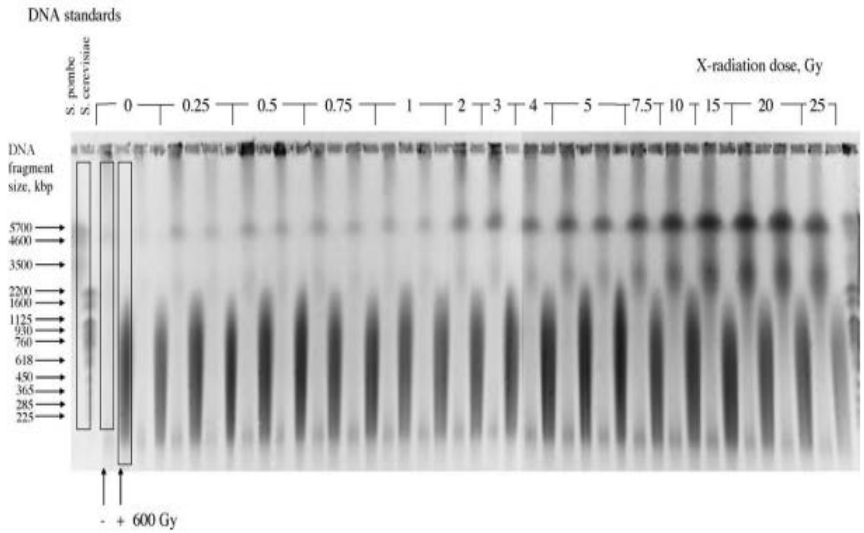


Fig. 3. Negative of a typical PFGE image. Human G1-lymphocytes were irradiated with increasing doses of X-rays at 0–4 °C, embedded in agarose plugs and lysed. Electrophoresis conditions were as described in Section 2.6. Given are molecular sizes of the DNA standards enclosed in the run. Arrows ± 600 Gy indicate samples that were additionally subjected (or not) to a 600 Gy dose of γ-rays before electrophoresis. Areas of analysis for the DNA standards and the samples are within the marked boxes.

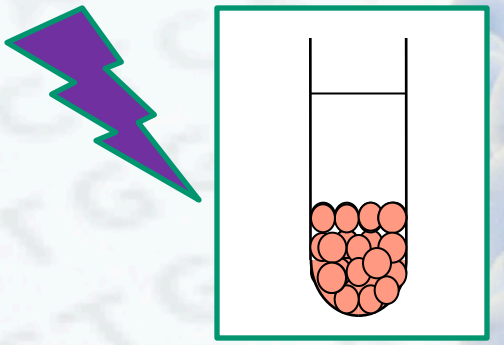
Advantages:

- high resolution in estimating the size of large DNA fragments (up to 10 Mb).
- ability to estimate the fragments resulting from clustered damage.

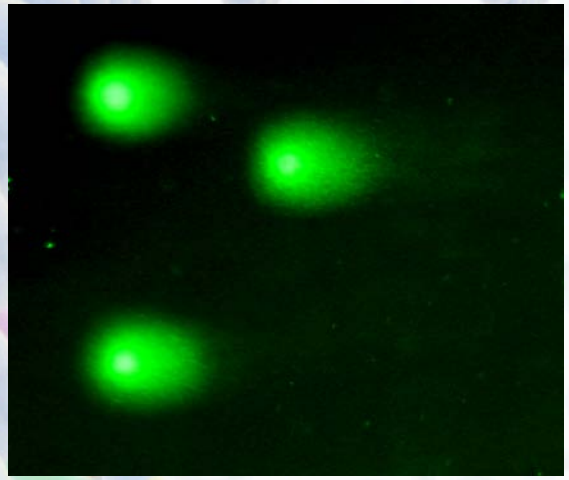
Disadvantages:

- very low sensitivity (> 200 DNA DSBs/cell);
- DNA fragmentation is estimated in the total cell population without regard for its heterogeneity

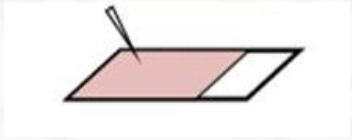
DNA comet assay



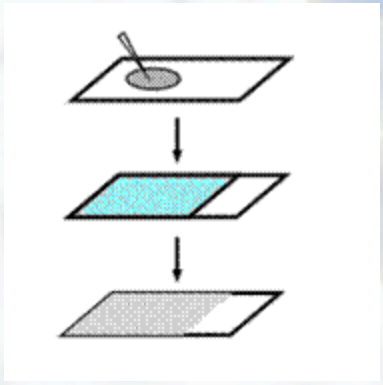
cellular suspension



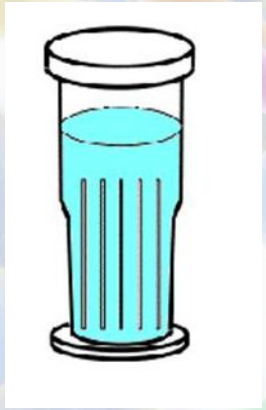
DNA comets visualization and analysis



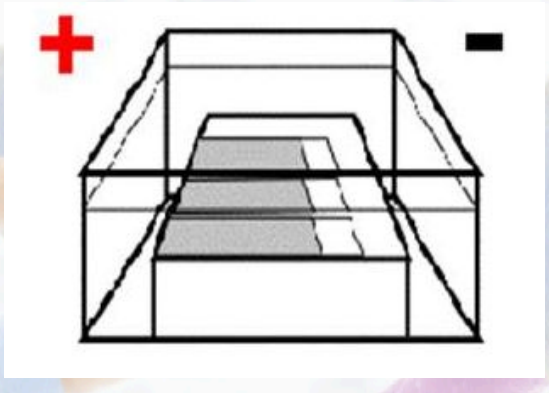
DNA staining



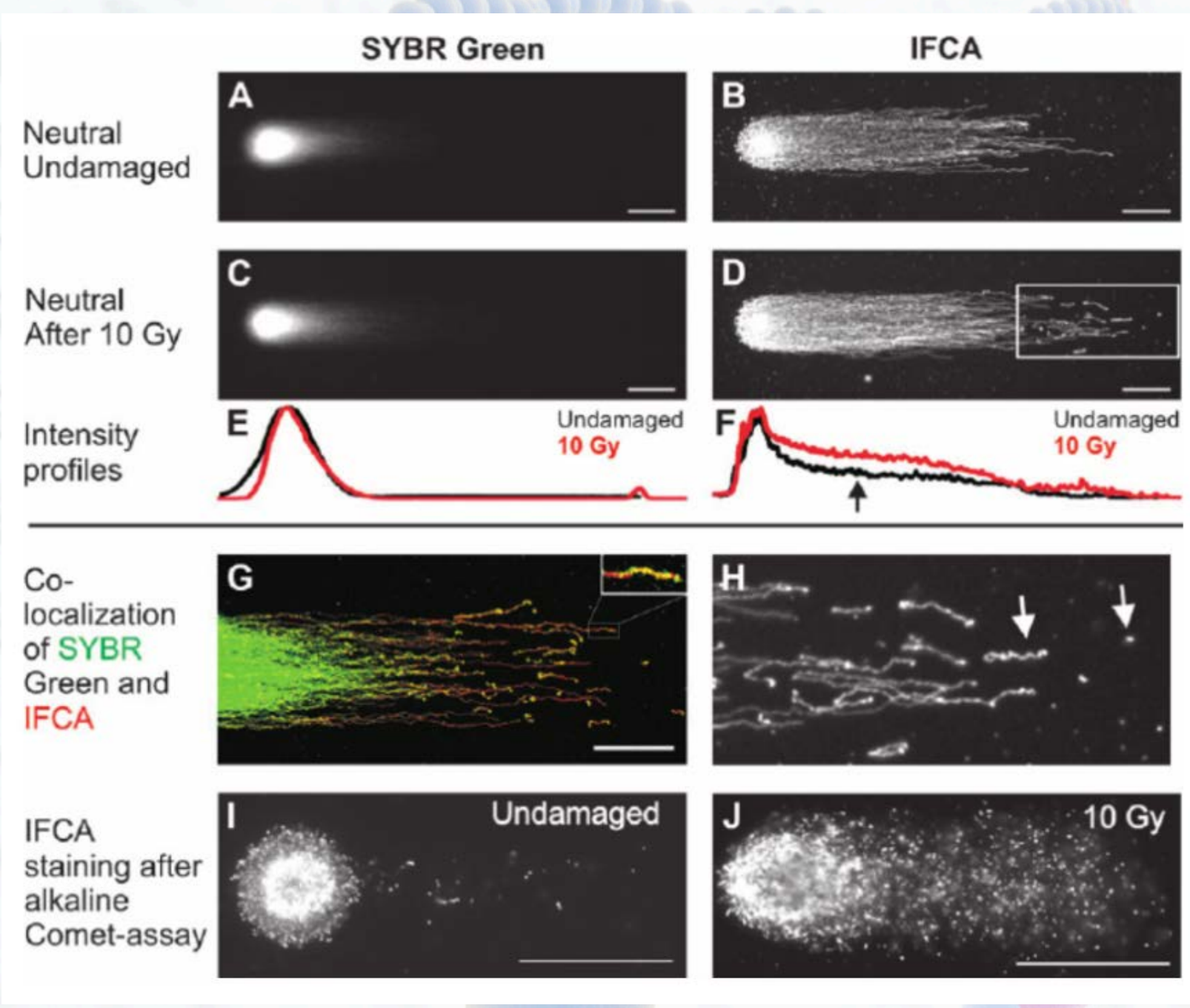
cells immobilization into agarose



cell lysis



electrophoresis



Grigaravicius et al., 2010

Advantages:

- single cell analysis
- relatively high sensitivity (~ 50-100 DNA DSBs/cell).

Disadvantages:

- specificity is controversial.

TUNEL assay

(Terminal deoxynucleotidyl transferase dUTP nick end labeling)

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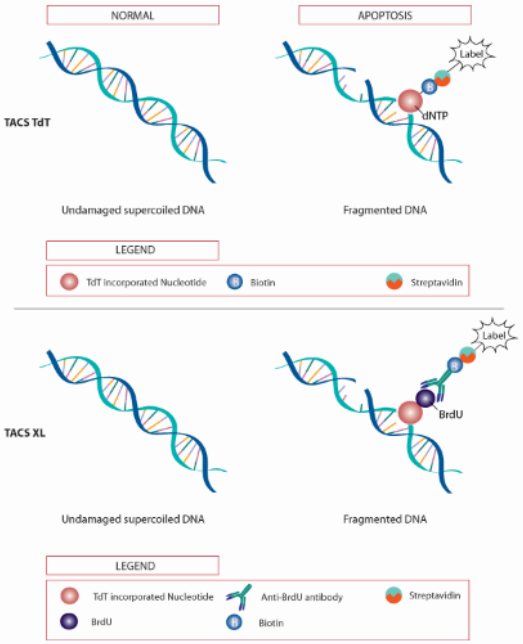
Home » Technical Resources » TUNEL Assay Principle

TUNEL Assay Principle



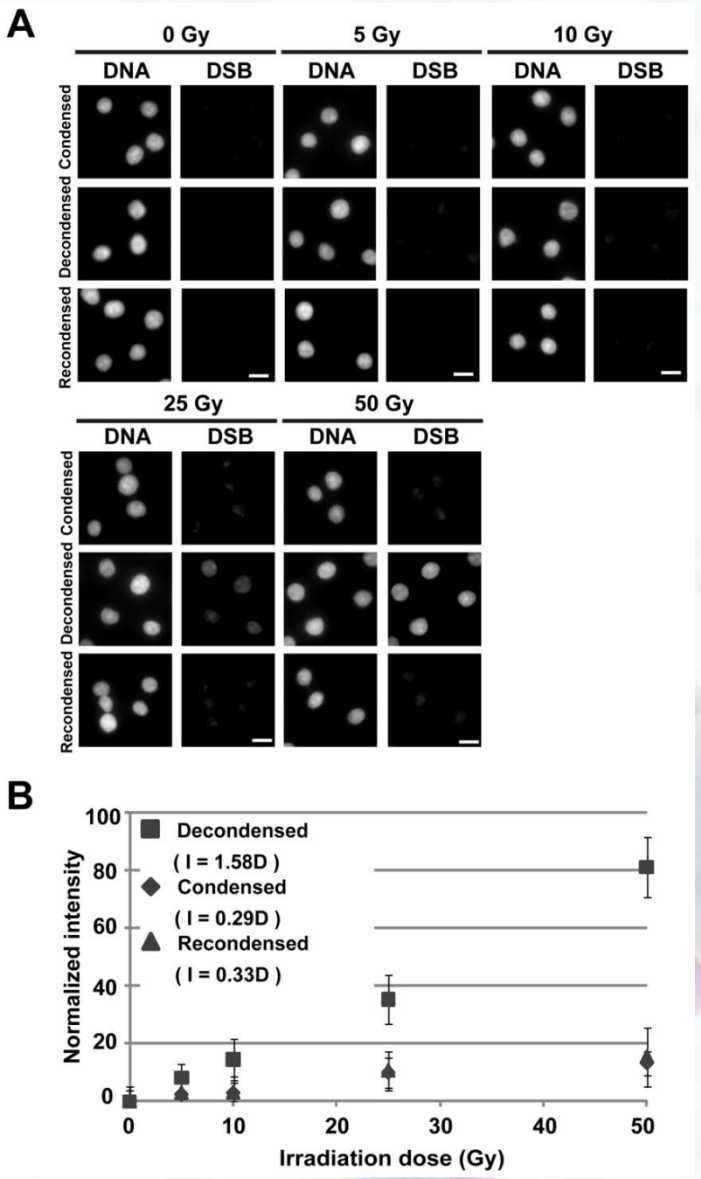
DNA fragmentation represents a characteristic hallmark of apoptosis. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) is an established method for detecting DNA fragments.

The TACS® TdT kits contain a highly purified form of the TdT enzyme for the enzymatic incorporation of biotinylated nucleotides. Biotin labeling is achieved using Streptavidin-horseradish peroxidase, and colorimetric substrates diaminobenzidine (DAB) or TACS Blue Label™.

TACS XL® kits embody a novel approach for the detection of apoptosis. This assay is based on incorporation of biotinylated nucleotides conjugated to bromodeoxyuridine (BrdU) at the 3' OH ends of the DNA fragments that form during apoptosis. This detection system utilizes a biotin conjugated anti-BrdU antibody and streptavidin-horseradish peroxidase. TACS XL kits are available with colorimetric substrates diaminobenzidine (DAB) or TACS Blue Label.

Related Information

- TUNEL Assays



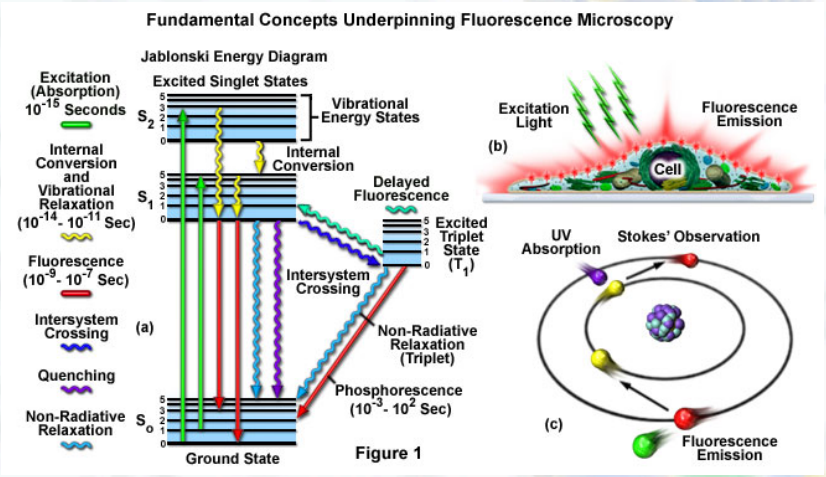
Advantages:

- single cell analysis

Disadvantages:

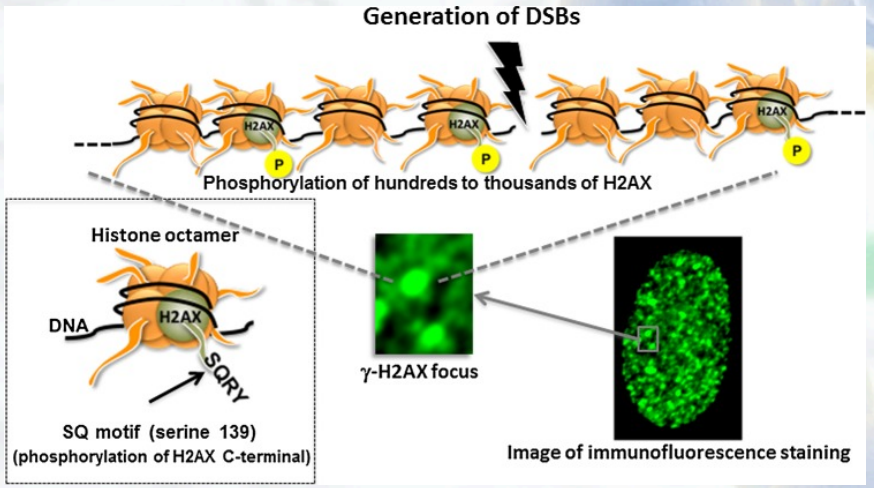
- low sensitivity (> 200 DNA DNA / cell)

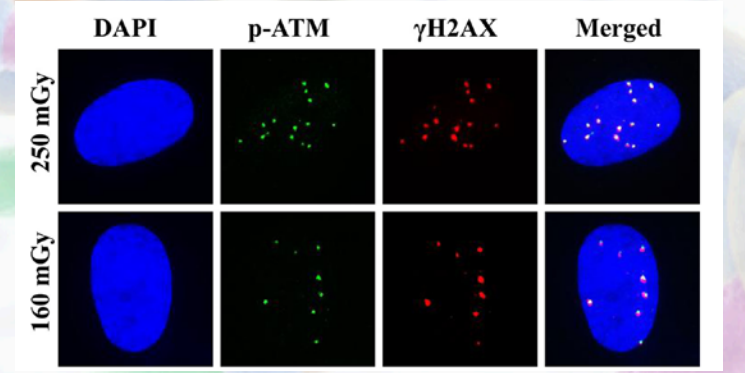
Immunocytochemical analysis



http://zeiss-campus.magnet.fsu.edu/articles/basics/fluorescence.html

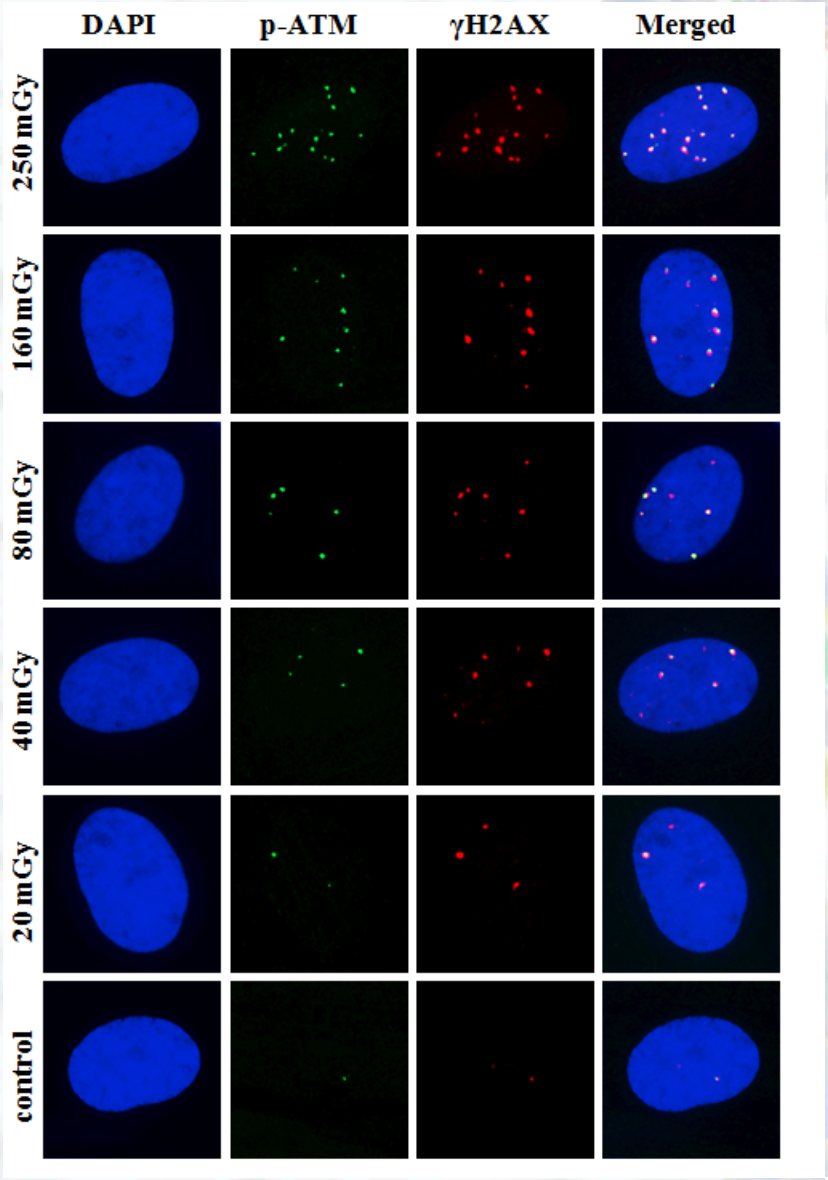
Immunofluorescent labeling of proteins involved in processing of a single DNA double-strand break (DSB) makes possible microscopic visualization of the DNA repair structures as distinct spots or foci that typically correspond to individual DSB. This allows for very accurate and sensitive indirect quantification of DNA DSBs and their repair, thus facilitating examination of molecular mechanisms of the repair process.

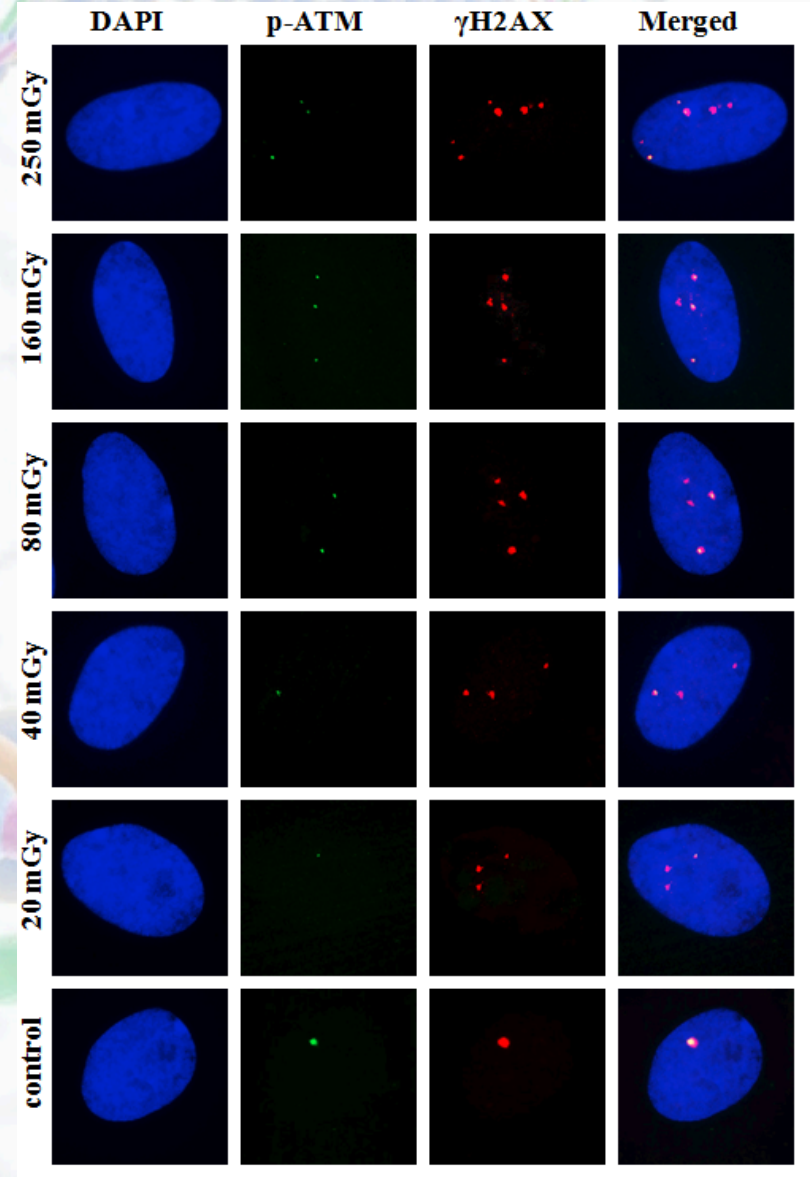




Osipov et al., 2015

30 min after irradiation — Low dose effects — 4 h after irradiation

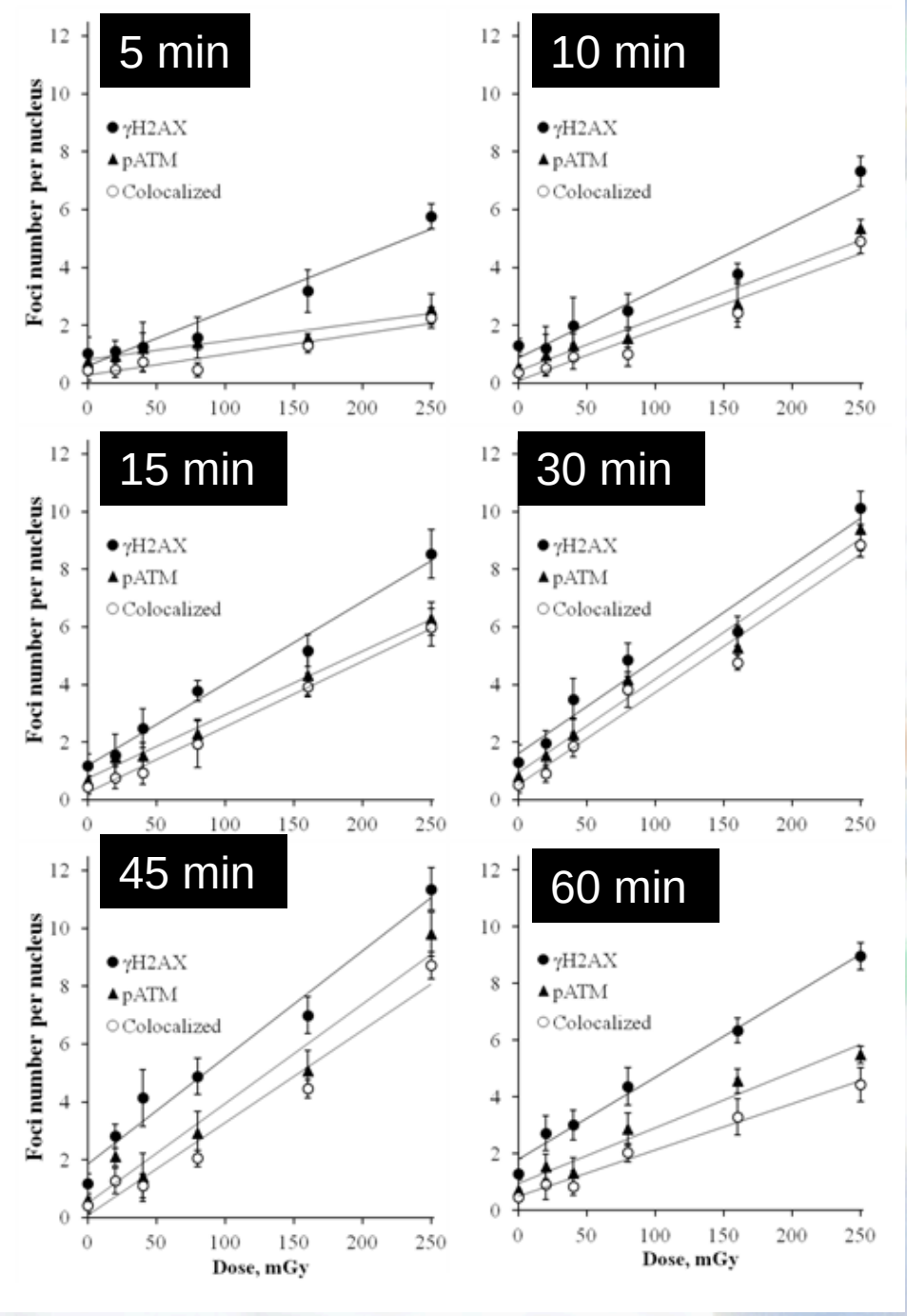


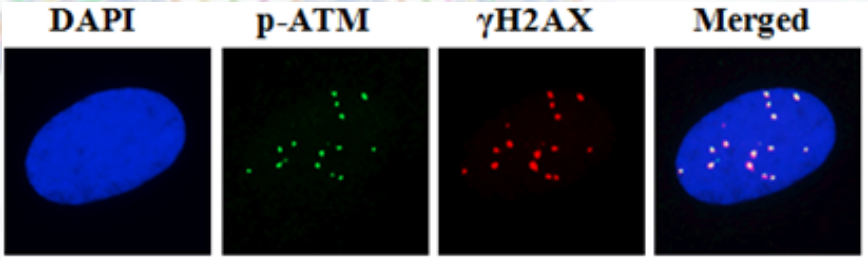


www.impactjournals.com/oncotarget/ Oncotarget, Vol. 6, No. 29

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

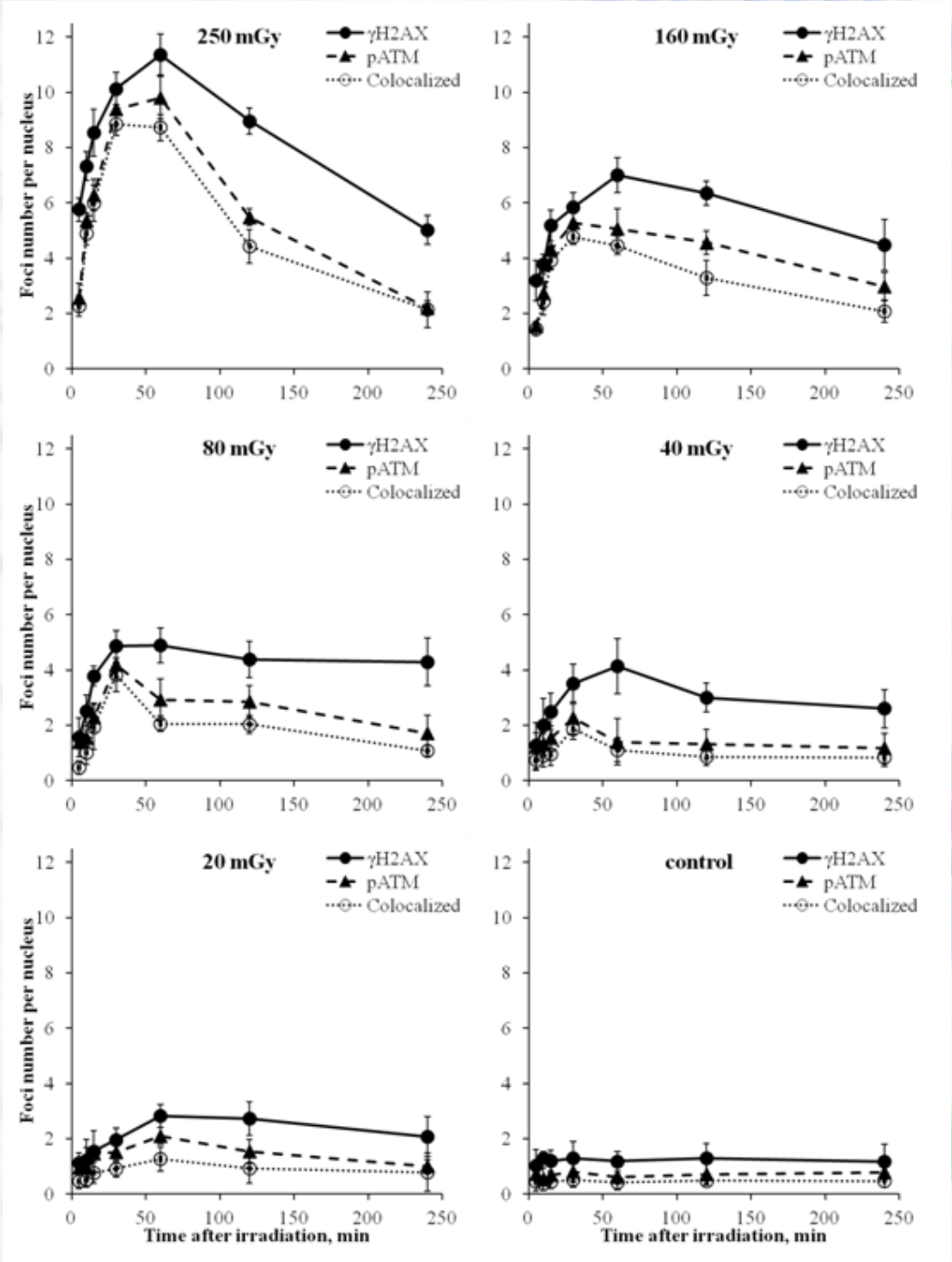
Andreyan N. Osipov[1,2,3,4], Margarita Pustovalova[1,2], Anna Grekhova[1,5], Petr Eremin[1], Natalia Vorobyova[1,3], Andrey Pulin[1], Alex Zhavoronkov[4,6,7], Sergey Roumiantsev[3,4,8], Dmitry Y. Klokov[9], Ilya Eremin[1]

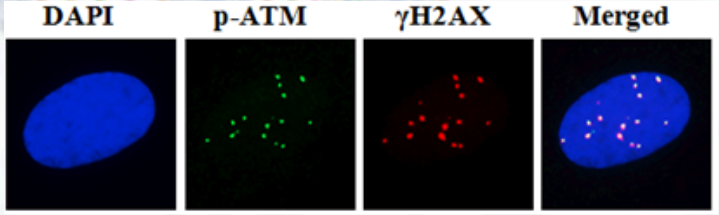




Radiation dose-responses for γH2AX and pATM foci in MSCs. Cells were exposed to X-irradiation at various indicated doses and fixed at 5 min (A), 10 min (B), 15 min (C), 30 min (D), 60 min (E) and 120 min (F). Number of foci for each protein and the number of colocolized foci were quantified and mean values of three independent experiments ± SD are shown on the graphs.

Osipov et al. Oncotarget. 2015. 6(29) 27275-27287.



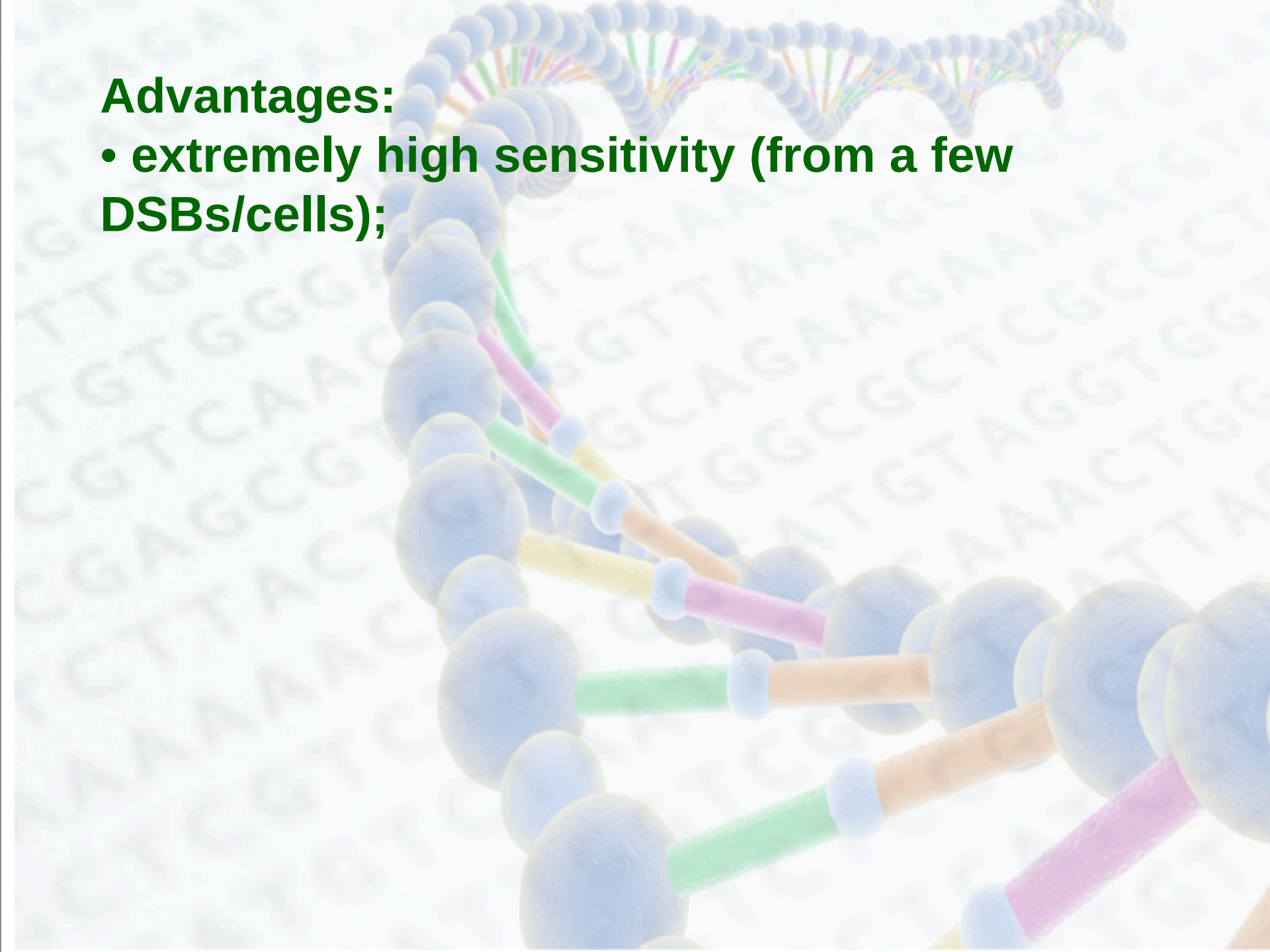


Kinetics of γH2AX and pATM foci induced in MSCs. Cells were exposed to 250 mGy, 160 mGy, 80 mGy, 40 mGy, 20 mGy or left untreated and fixed at various indicated time-points after irradiation up to 240 min. Number of γH2AX and pATM foci were quantified, as well as their co-localization and mean values from three independend experiments ± SD were plotted.

Osipov et al. Oncotarget. 2015. 6(29) 27275-27287.

Advantages:

- **extremely high sensitivity (from a few DSBs/cells);**



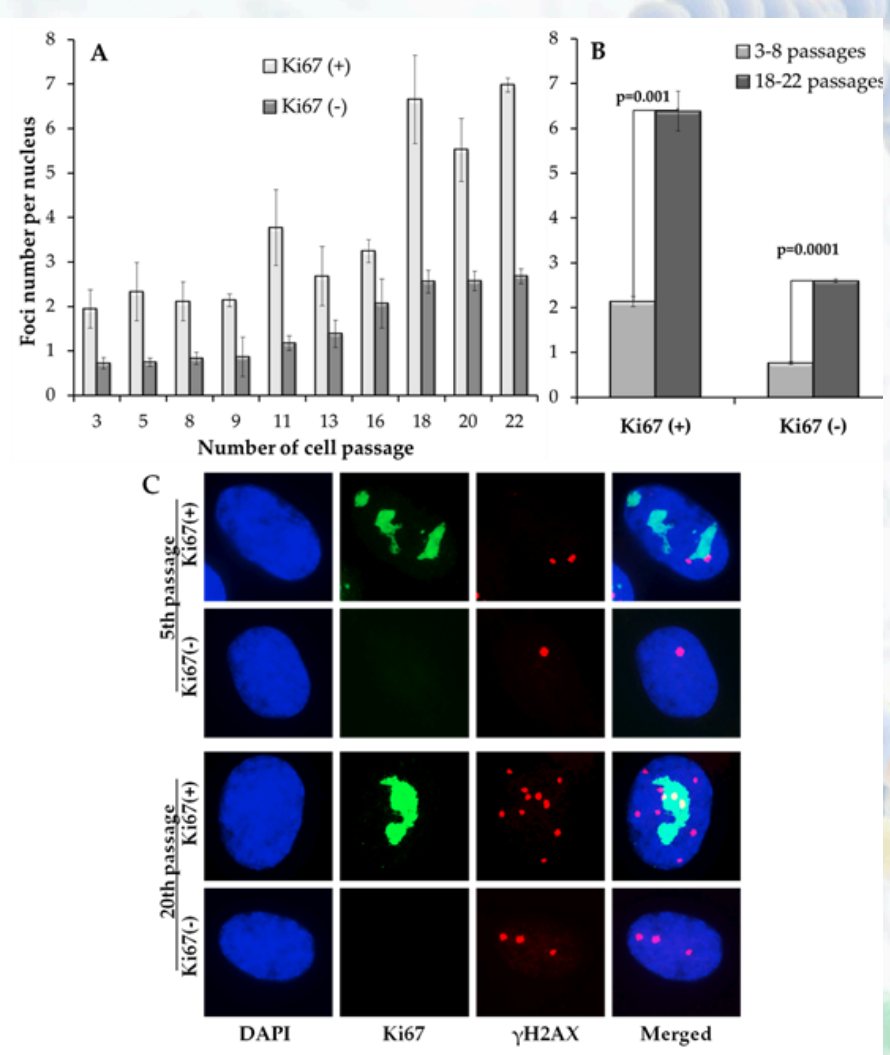
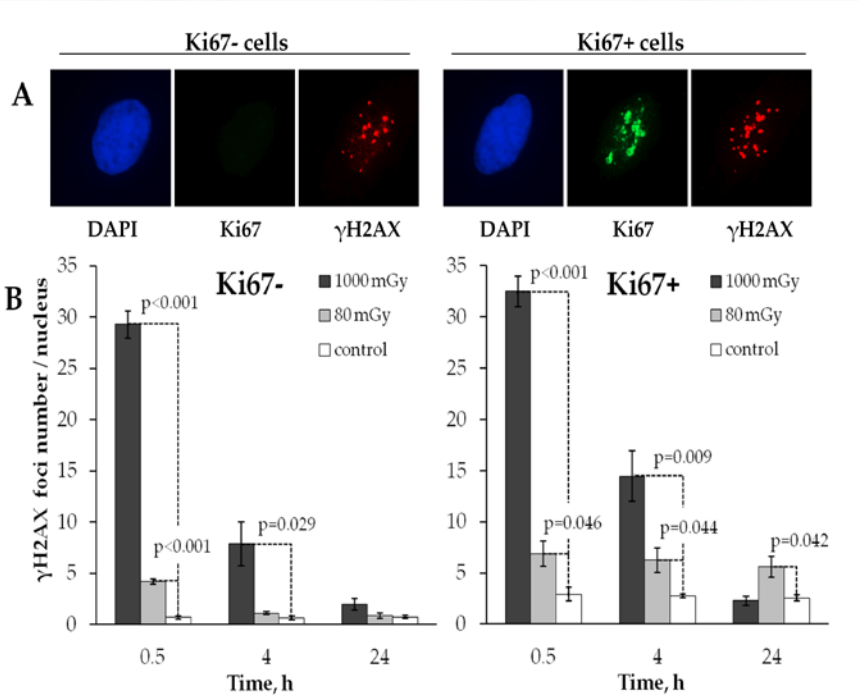


Figure 2. Differential immunocytochemical analysis of γH2AX foci in proliferating (Ki67(+)) and resting (Ki67(-)) cells: (**A**) Changes in the γH2AX number in Ki67(+) and Ki(-) cells on 3-22 passages (**B**) Comparative analysis of γH2AX in Ki67(+) and Ki(-) cells on early (3-8) vs. late (18-22) passages; (**D**) Representative immunofluorescent microphotographs of MSC showing Ki67 (green), γH2AX (red) foci and their co-localization (yellow) at passage 5 and 20. Nuclei were counterstained with DAPI.



www.aging-us.com AGING 2017, Vol. 9, No. 11

Research Paper

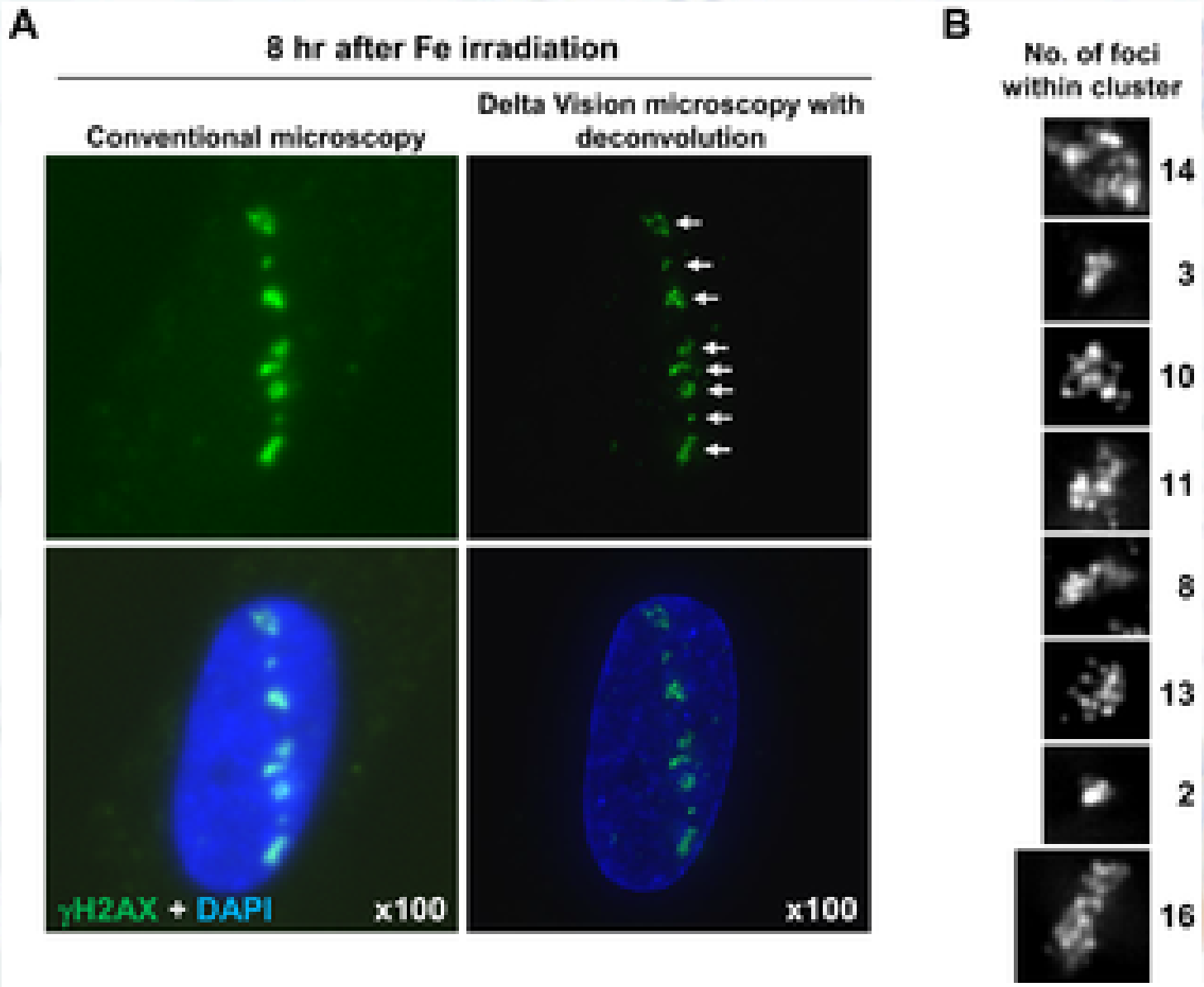
Residual γH2AX foci induced by low dose x-ray radiation in bone marrow mesenchymal stem cells do not cause accelerated senescence in the progeny of irradiated cells

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]

Osipov et al. Aging. 2016

Advantages:

- **extremely high sensitivity (from a few DSBs/cells);**
- **differentiated analysis of heterogeneous cell populations according to various parameters (proliferation status, cell cycle etc.);**



OPEN ACCESS Freely available online

PLOS ONE

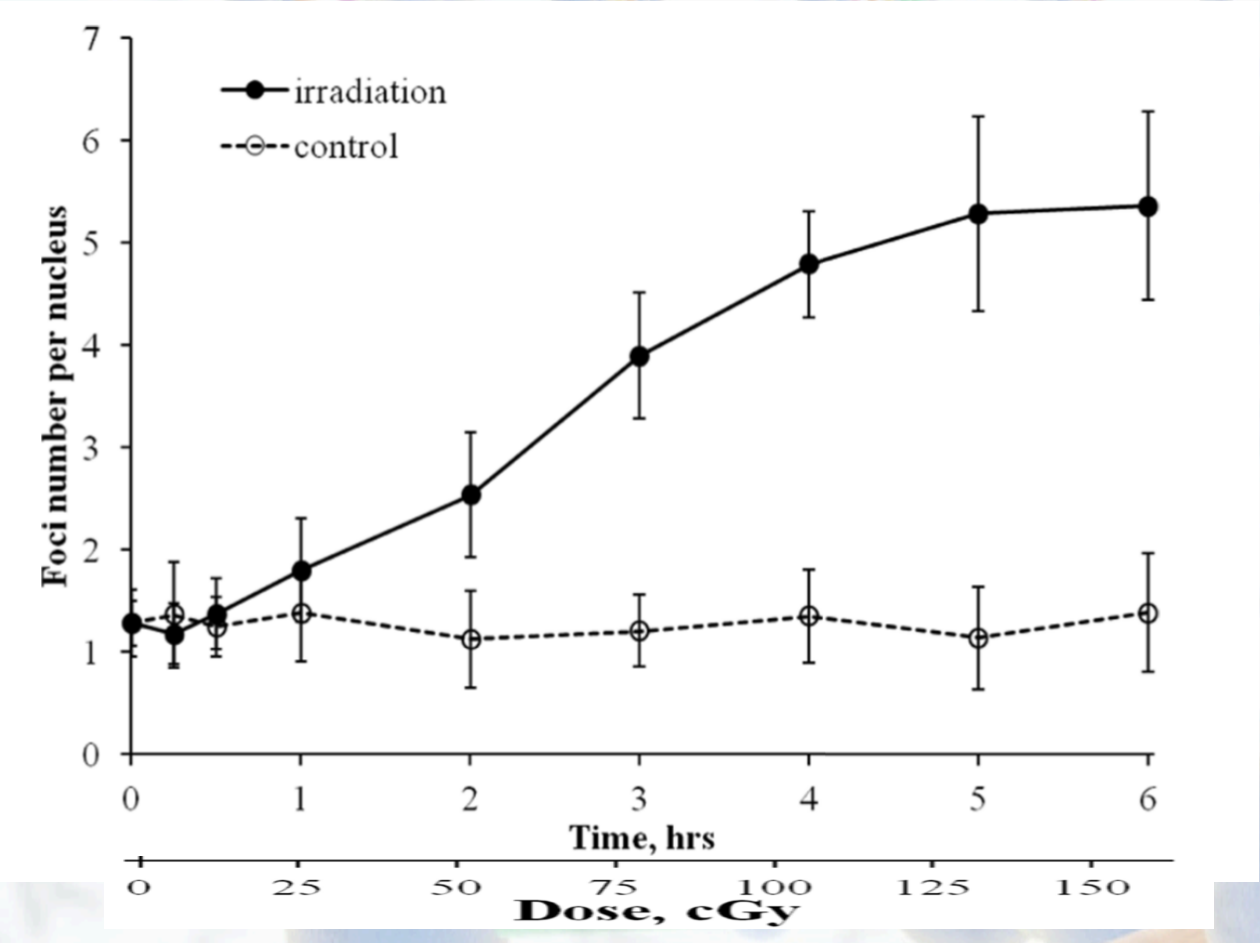
Visualisation of γH2AX Foci Caused by Heavy Ion Particle Traversal; Distinction between Core Track versus Non-Track Damage

Nakako Izumi Nakajima[1], Holly Brunton[2], Ritsuko Watanabe[3], Amruta Shrikhande[2], Ryoichi Hirayama[1], Naruhiro Matsufuji[1], Akira Fujimori[1], Takeshi Murakami[1], Ryuichi Okayasu[1], Penny Jeggo[2]*, Atsushi Shibata[2]*¤

Advantages:

- **extremely high sensitivity (from a few DSBs/cells);**
- **differentiated analysis of heterogeneous cell populations according to various parameters (proliferation status, cell cycle etc.);**
- **spatial distribution analysis;**

Formation of RAD51 foci in diploid normal human fibroblasts during continuous exposure to X-ray radiation at a dose-rate of 4.5 mGy/min.



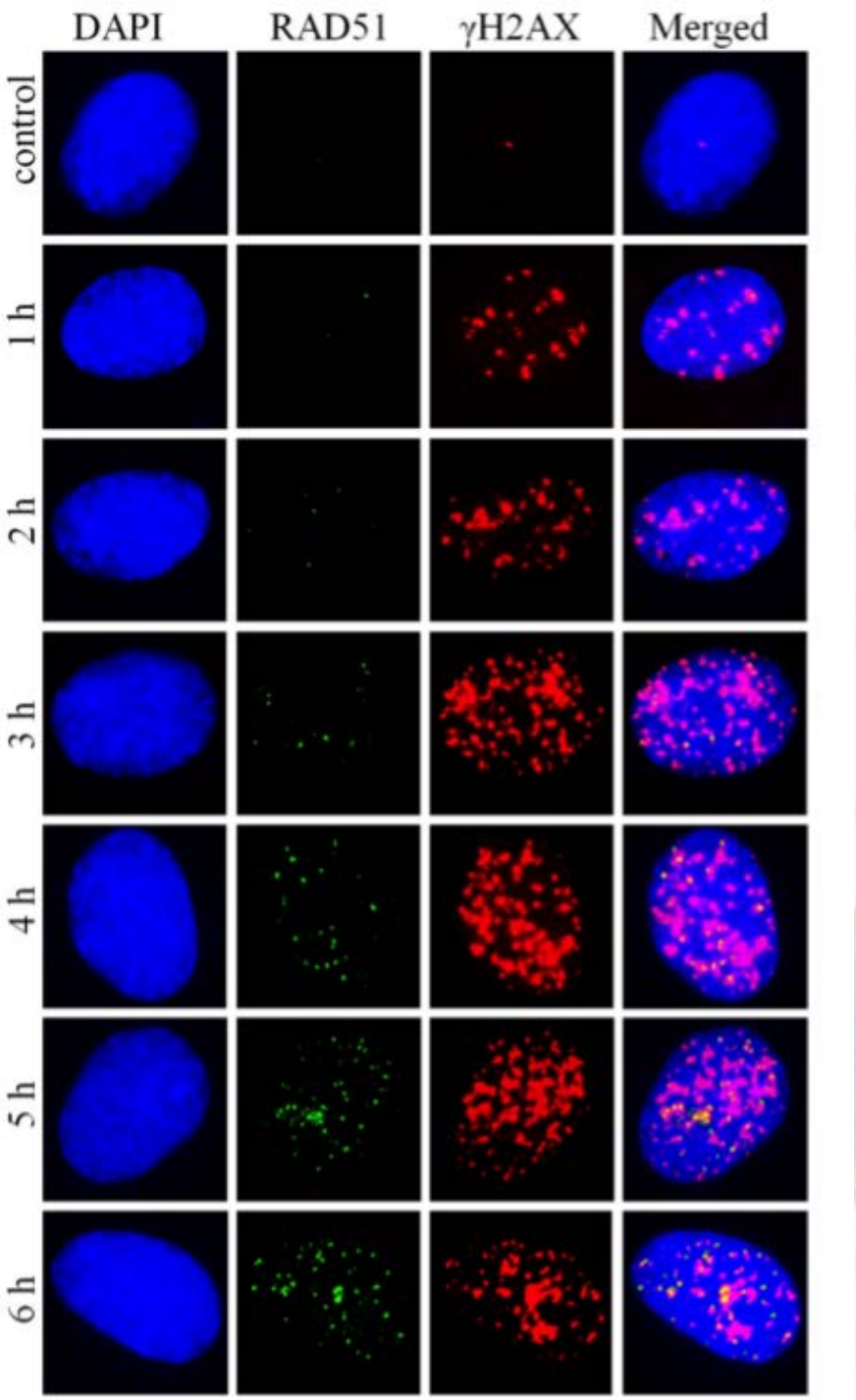
RAD51 foci were quantified using immunofluorescence microscopy. Two hundred cells per data point were analyzed per experiment. Means calculated from three independent experiments ± standard errors are shown.

www.impactjournals.com/oncotarget/ Oncotarget, Vol. 6, No. 29

Activation of homologous recombination DNA repair in human skin fibroblasts continuously exposed to X-ray radiation

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

Representative microphotographs of RAD51 and γH2AX foci formed in diploid normal human fibroblasts upon exposure to X-ray radiation at a dose-rate of 4.5 mGy/min.



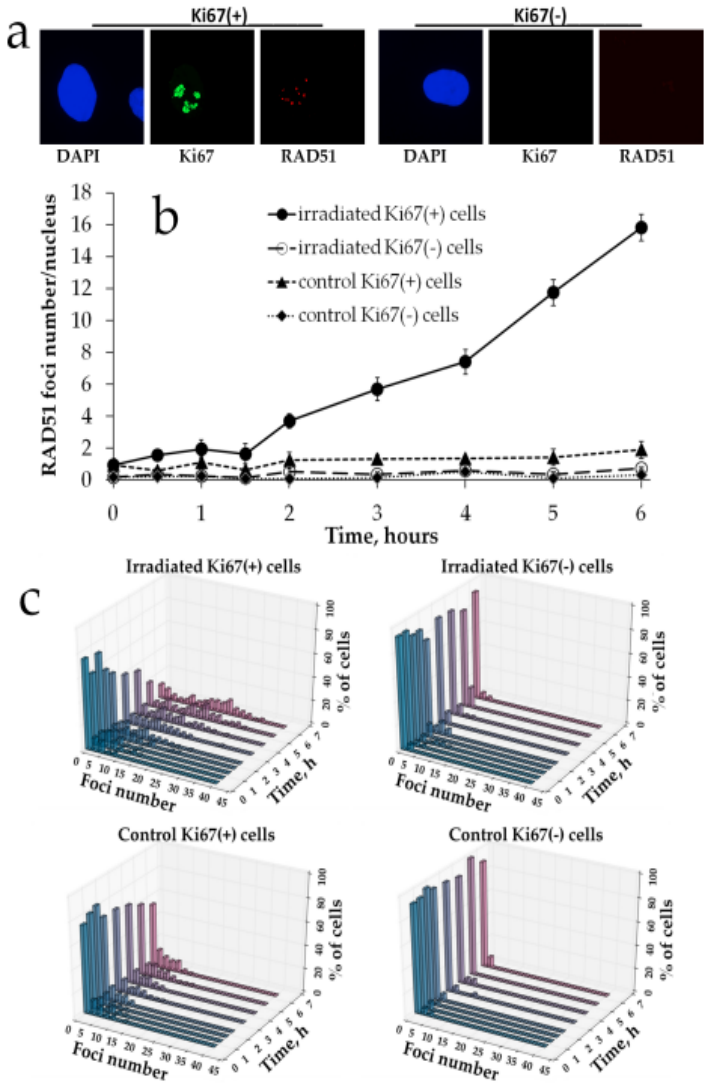


Figure 4: Rad51 foci formation in proliferating vs. resting MSCs exposed to prolonged X-ray irradiation. **(a)** Representative microphotographs of immunofluorescently stained irradiated MSCs showing Ki67 (green) and Rad51 foci (red). DAPI counterstaining is shown in blue. **(b)** Quantification of Rad51 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.

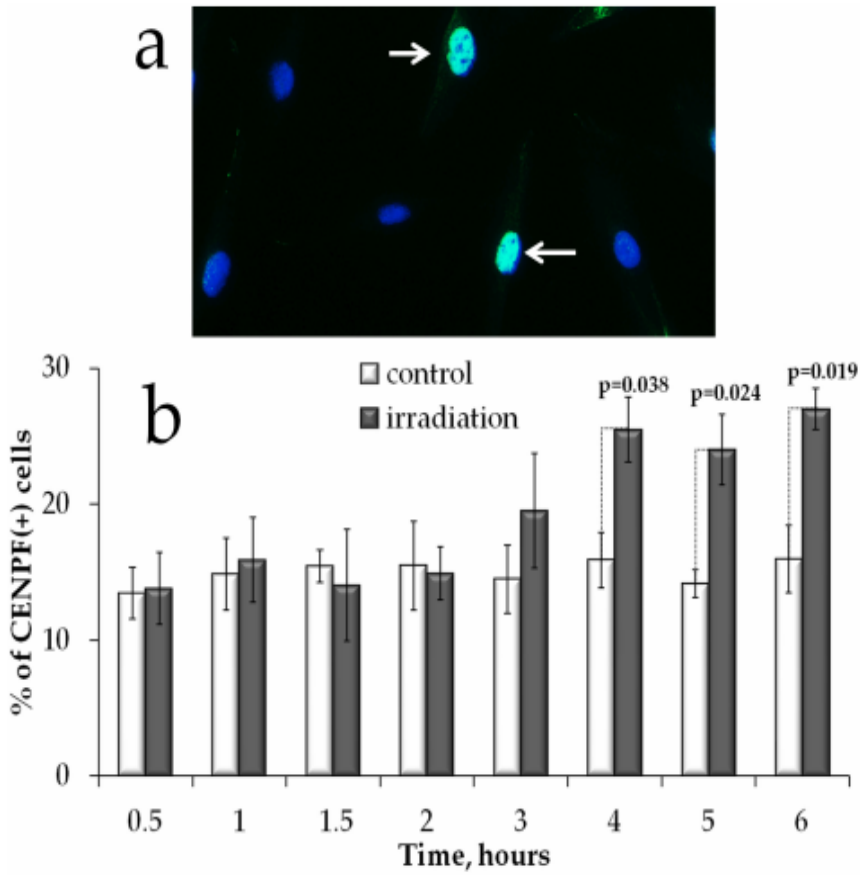


Figure 6: S/G2 cell cycle phases changes in MSCs exposed to prolonged irradiation. **(a)** Representative microphotographs of immunofluorescently stained irradiated MSCs showing CENPF (green) DAPI counterstaining (blue). **(b)** Quantification of CENPF+ cells in cultures exposed to prolonged (270 mGy/h) 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.

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]

Advantages:

- **extremely high sensitivity (from a few DSBs/cells);**
- **differentiated analysis of heterogeneous cell populations according to various parameters (proliferation status, cell cycle etc.);**
- **DNA damage spatial distribution analysis;**
- **DNA repair mechanism studies.**

19-14-00151

RSF Russian Science Foundation

RSF Project «Molecular and cellular effects of ultrashort pulsed radiation»

Russia + **Armenia**

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CANDLE

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Institute of MOLECULAR BIOLOGY
National Academy of Sciences of Armenia

The main task of the project is analysis of radiobiological effects after irradiation of human normal and tumor cells with subpicosecond pulses of accelerated electron beams. The large scale systematic studies with analysis of key molecular and cellular parameters (induction of DNA damage and repair; cell cycle and proliferation arrest; cell death) are planned. The results obtained will help to select the strategy of further research of the possibility of application of ultrashort pulse irradiation for the development of new technologies of radiation therapy of malignant tumors in humans.

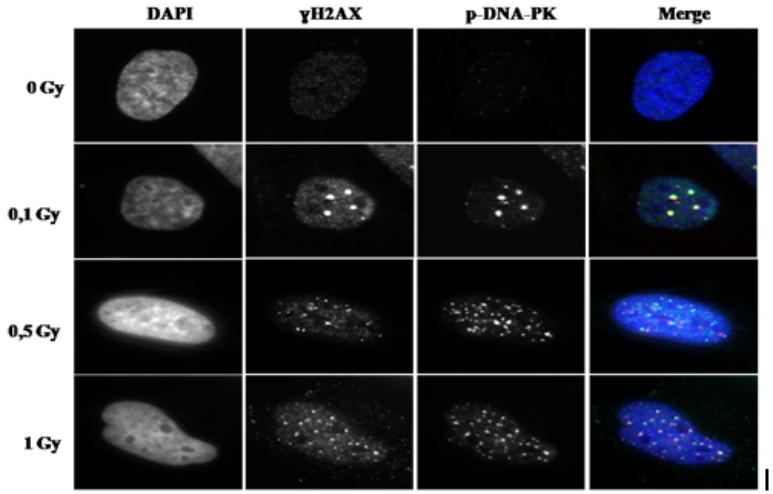

Figure 1. Representative images of γH2AX and p-DNA-PK foci and their co-localization at 1h post-irradiation in MRC5 cell line after irradiation with ultrashort/ultrafast electron beam

Babayan et al., in preparation

Thank you for your time and attention!!!