Radiobiological Effects of Ultrashort Pulsed Electron Beam Irradiation In Vitro and In Vivo

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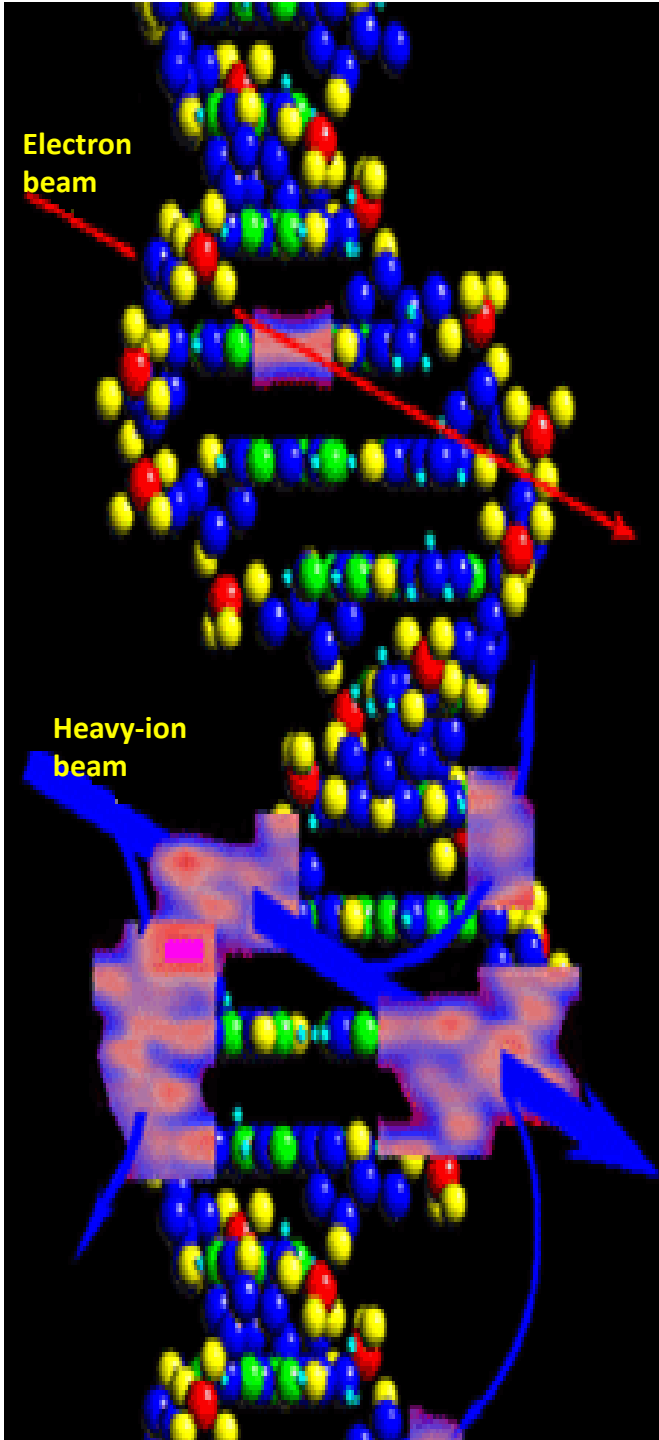
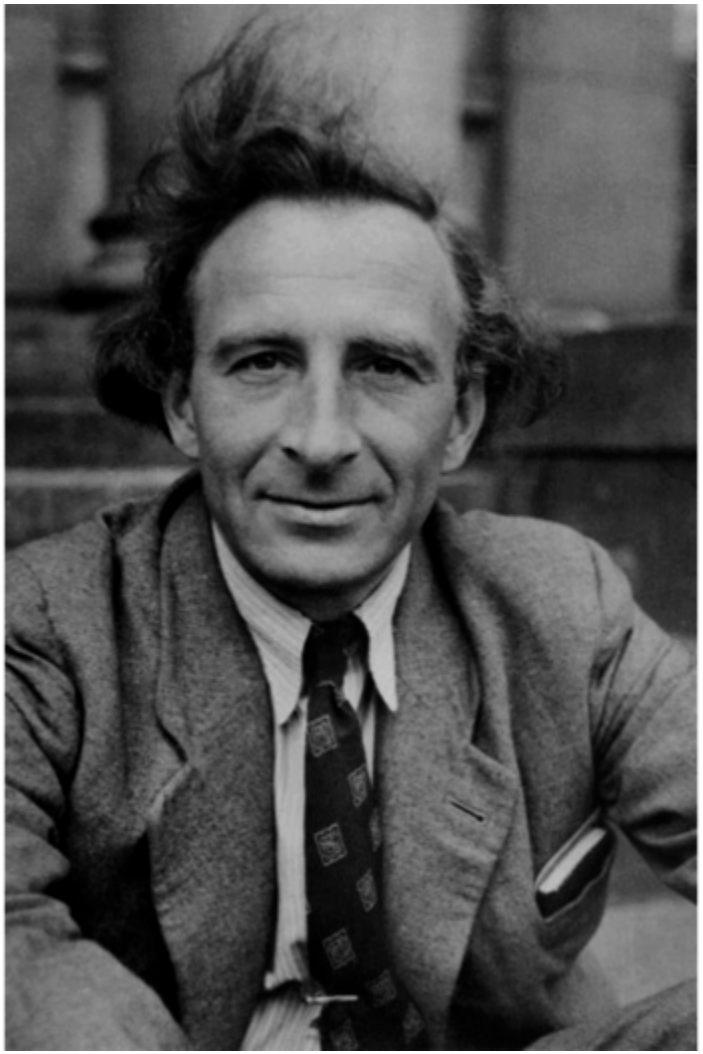




Nikolay Timofeev-Ressovsky
(1900-1981)

Timofeeff, along with his German co-workers Karl G.Zimmer and Max Delbruck, set out the target-or hit-theory based on this analogy. The classic "three-man paper" "On the Nature of Gene Mutation and Gene Structure" (1935) **describing their work inspired Erwin Schrodinger to deliver his 1943 course of lectures, later published as the book - What Is Life?,** which helped draw many physicists to molecular biology.

Timofeeff's principal discovery was his observation of a **linear relation between the total radiation dose and the number of mutations.** Whether the dose was administered in a single shot, in several fractions or continuously at a low level over an extended period appeared irrelevant.
The intensity of the dose did not affect the number of mutations produced.
He also **found no minimum dose below which mutations were not generated.**

He suggested that **X rays produce mutations much like bombs hitting targets**. **In the target model**, an **X-ray photon expels electrons from atoms**. These unbound electrons hit other atoms, dislocating more electrons, and so on. The free electrons eventually settle in the electron shells of other atoms**. In** this way, an X ray creates positively charged ions (atoms missing electrons) and negatively charged ones (atoms having a surplus of electrons).
One ionization in a gene causes a mutation.

Timofeeff and his collaborators set out to estimate the size of a single gene by calculating the number of ionizations produced in a certain volume of tissue and by recording the increased number of mutations of a particular gene in that tissue.

Timofeeff and his co-workers found the gene to be a sphere: one to 10 microns!
Not only for international, but also for Armenian radiobiology!

A. ALIKHANYAN
National Laboratory

Artem Alikhanyan
(1908 – 1978)

The scientific reserve of the scientific team

The results of 25-year studies of radiation mutagenesis on samples of patients irradiated during the liquidation of the Chernobyl accident have been published in international journals.

Within the framework of long-term cooperation with German geneticists, the genetic effects of neutron radiation in cells of cancer patients have been studied.

Now we have general research laboratory with German geneticists and international grant with Russian radiobiologists.

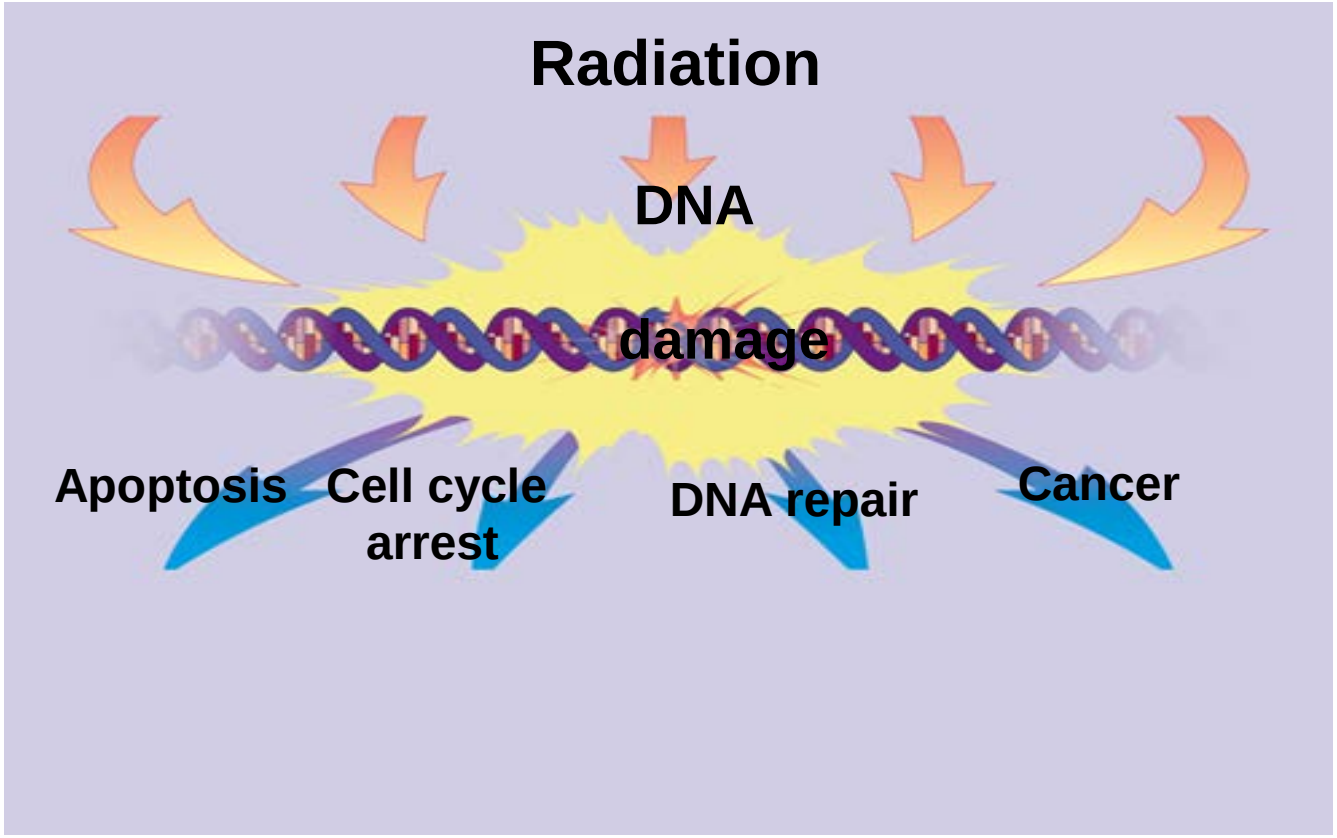
For geneticists, the most important direction in radiobiology is the DNA damage, induced by different types of radiation, including ultrafast electron beams.

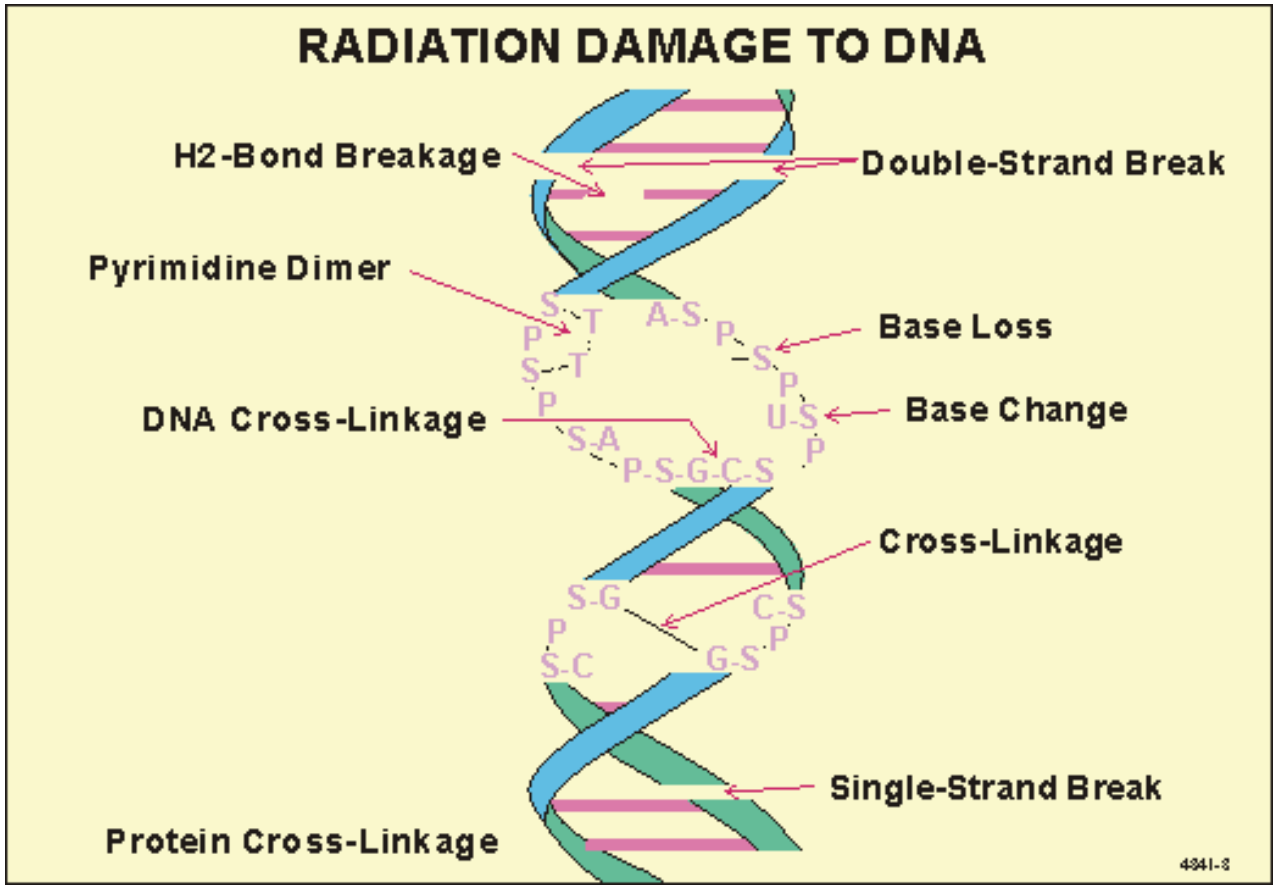
The damaged DNA can be repaired, and if repair is not successful, then:

-cells can die by apoptosis.

-arrest in cell cycle can be induced.

-DNA damage can lead to cancer.




RADIATION DAMAGE TO DNA
H2-Bond Breakage
Double-Strand Break
Pyrimidine Dimer
Base Loss
DNA Cross-Linkage
Base Change
Cross-Linkage
Single-Strand Break
Protein Cross-Linkage
4341-8

The role of radiation therapy and DNA damage control is important, because about 50 % of cancer cases are treated with radiation therapies (possibly in combination with surgery and/or chemotherapy).

Among these treatments, more than 50 % use RF-driven linear accelerators of electrons (RF-Linac).

- **Other techniques include internal radiation (brachytherapy) and proton-ion beams (hadrotherapy).** In most cases electrons delivered by a RF-linac are not used directly on the tumor but converted into photons (hard X-rays) by bremsstrahlung through a suitable target.
- **In some case electrons are used directly, either to cure superficial tumors or in the Intra-Operative Radiation Therapy which can be applied during surgical operation of tumor.**

The laser based acceleration leads to pulses with an outstandingly high pulse dose rate close to the source which may result in an altered radiobiological response.

This has to be investigated first by in vitro studies with different tumor and normal tissue cell lines and followed by in vivo studies with animal irradiations.

Karsch et al. (2017) Towards ion beam therapy based on laser plasma accelerators.

Advantages and disadvantages of laser-based accelerators

Ultrahigh peak dose-rate (UHPDR)

The possibility of creating directed **ultra-short pulses of enormous dose rate** may allow the **precise dose-control induction of local effects on solid tumors with minimal exposure of normal tissues**

Up to now the in vitro and in vivo experiments were performed on

- **JETI** (Jena Titanium:Sapphire laser system)
  - UHPDR - **2.4×10^{9} Gy/sec**
  - pulse duration - **1×10^{-12} s**
- **ELBE** (Helmholtz-Zentrum Dresden – Rossendorf)
  - UHPDR - **1.6×10^{8} Gy/sec**
  - pulse duration - **5×10^{-12} s**

REGAE: The Relativistic Electron Gun for Atomic Exploration

Bunch length < 10^{-14} s

Average energy 5.6 MeV

Bunch charge 100fC

5J in 25fs@5Hz

AREAL

laser driven, photocathode RF gun based linear accelerator providing ultrashort electron pulses (sub-pico or femtosecond) with the electron energy in MeV domain

- pulse duration - **0.04×10^{-12} s**
- UHPDR - **1.6×10^{10} Gy/sec**

JETI and ELBE
~ $1\text{-}5 \times 10^{-12}$ s
~10^8-10^9 Gy/sec

In comparison to laser-based accelerators these facilities AREAL **produce electron pulses generated by UV laser and accelerated using high gradient RF resonator**, thus lead to

- precisely form beam parameters, provide high stability and reproducibility of electron beam and radiation characteristics

- shorter pulse duration (sub-pc vs. pc)

- **higher UHPDR (10^{10} Gy/sec vs. 10^8-10^9 Gy/sec)**
  (Tsakanov et al. 2016; Floettmann et al., 2013)

In comparison to conventional medical LINACs, where the beam stability parameters are satisfactory, however, **the electron pulses generated by LINACs have the lengths about hundreds of picoseconds**

The advantage
of laser-generated ultrafast electron beams
for biological and clinical application
is obvious, since they…

1. • **Typically feature a monoenergetic spectral profile and are better directed (less lateral spread) than other laser-driven ions.**

2. • **They have very high instantaneous dose delivery within a time interval shorter than many chemical reactions pulses of enormous capacity (up to kGy /s), but with small dose of 1 pulse. This allows the precise dose-control induction of local effects on solid tumors with minimal exposure of normal tissues.**

3. **•Engineering development activities in this field are far ahead of radiobiological research. There are only a few publications on the biological effects of pulsed ionizing radiation.**

4. • **It is assumed that accelerators of this type can improve radiation therapy of tumors.**

Radiobiological experiments:

Radiation Source: AREAL

AREAL:
Laser-driven radiofrequency gun-based linear pulsed electron accelerator
Electron beam:
Ultrafast – 3,6 MeV
Ultrashort – 400 fs

Application

- Potential alternative acceleration technology for ion radiotherapy
- More precise investigation of damage mechanisms

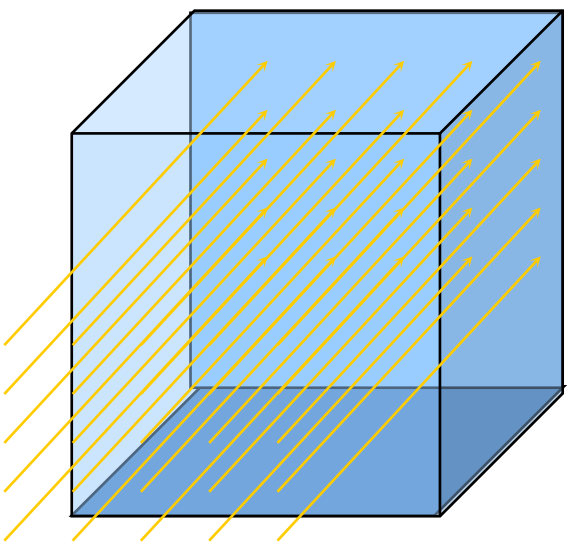
Radiobiological Endpoints

DNA damage and repair

Comparison of DNA damage response (DDR) after ultrashort pulsed electron beam and X-ray irradiation

Distribution of ionization in substance

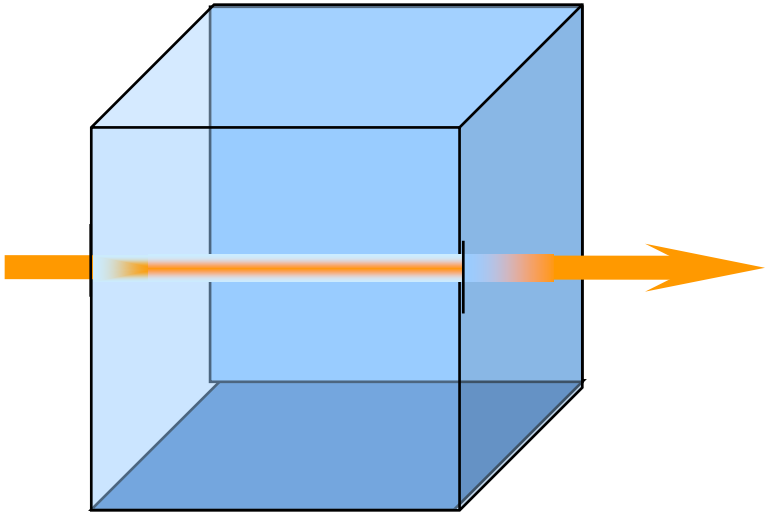
1 unit of dose



Low-LET radiation

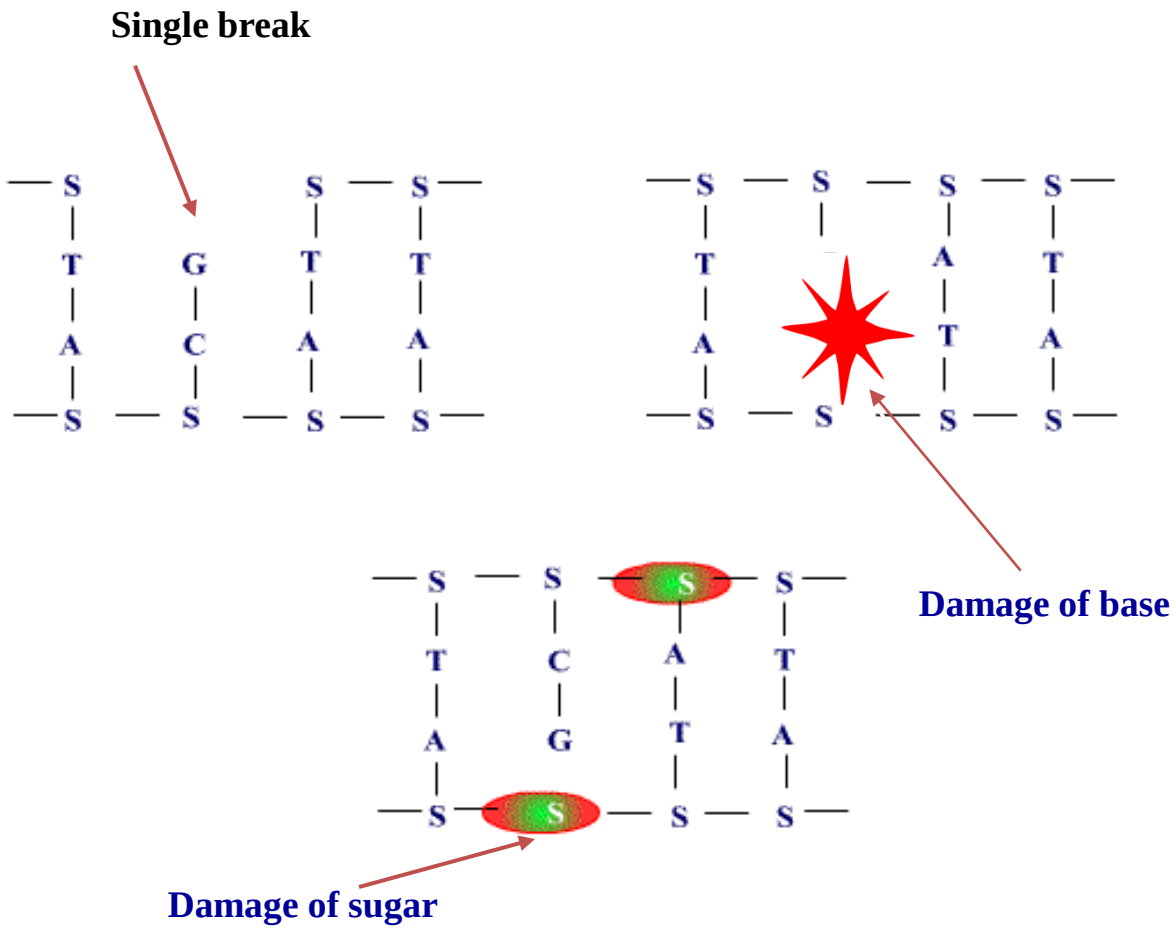
(such as X- and γ-rays or electron beam radiation)

1 unit of dose

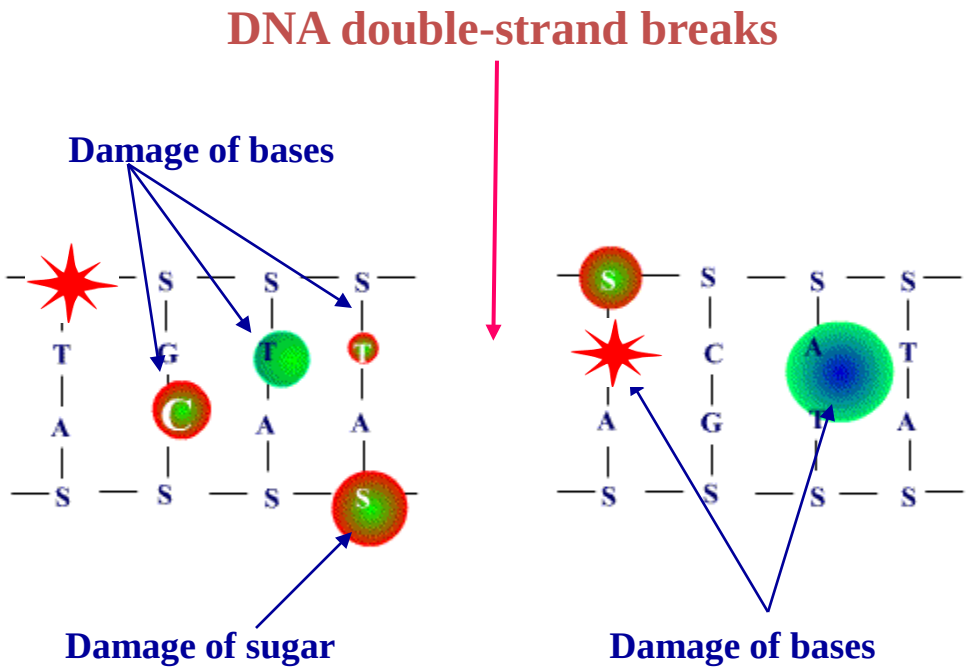


High LET radiation

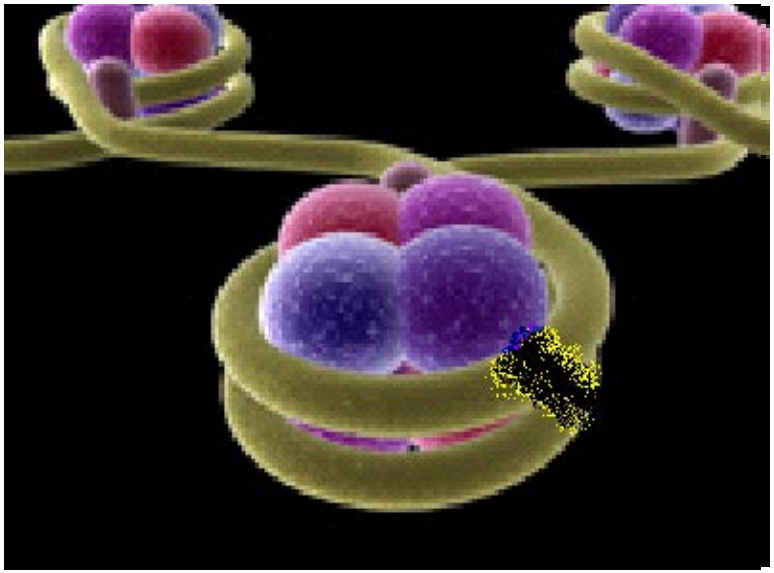
Single breaks of DNA



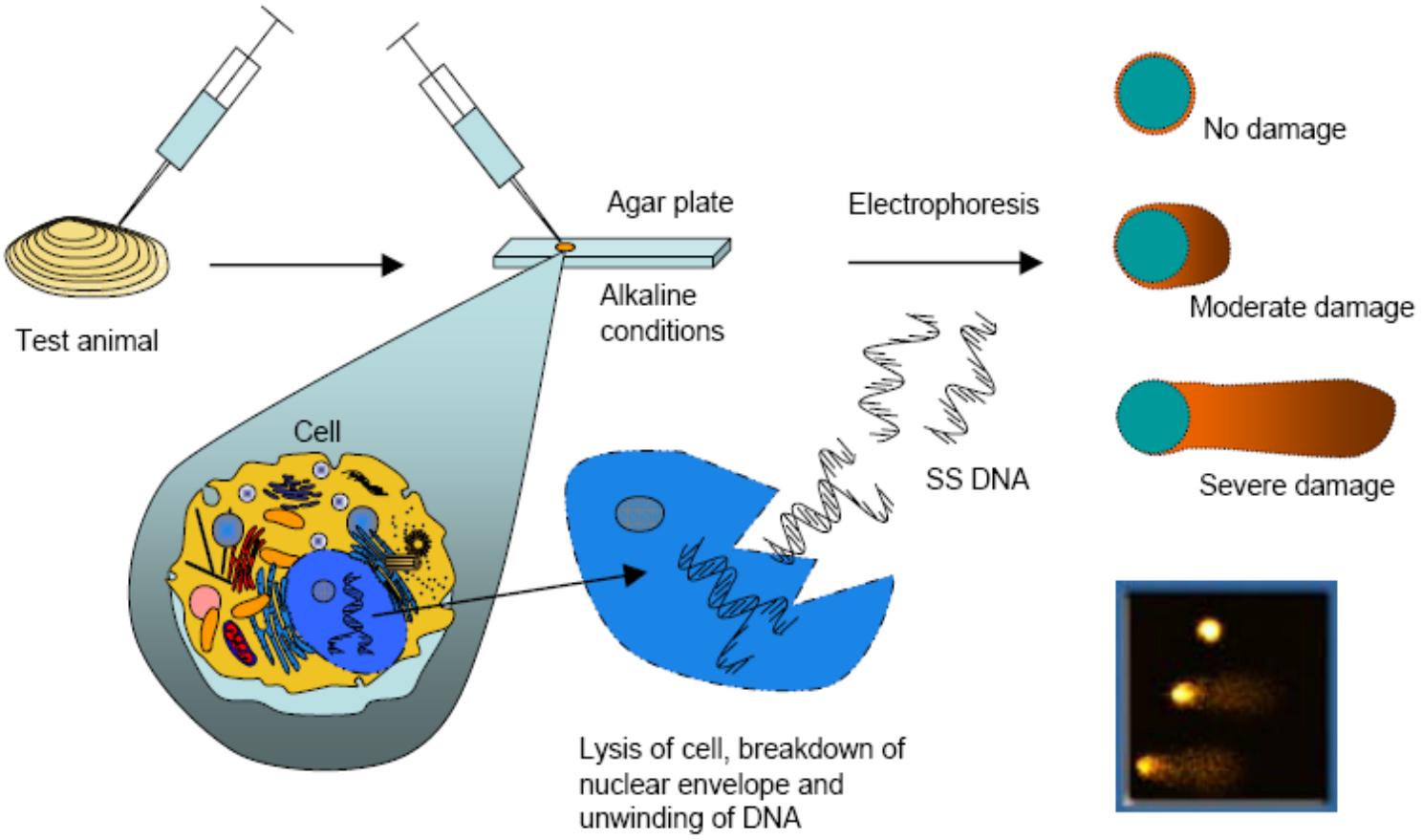
Cluster damage of DNA



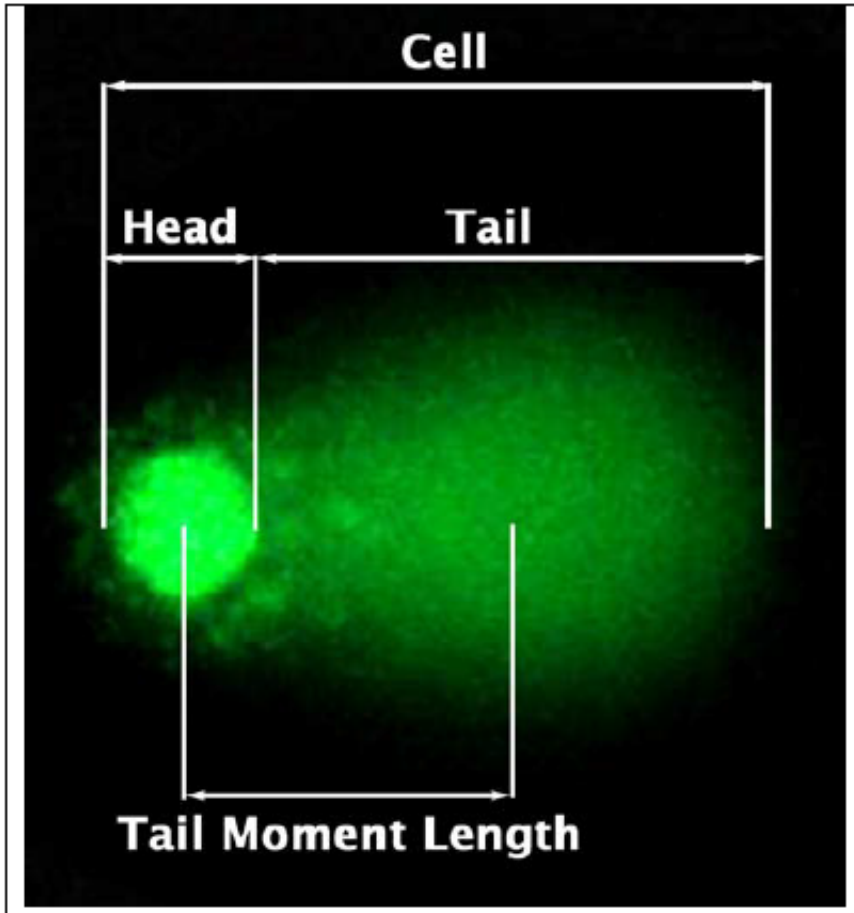
Cluster damage of DNA in nucleosome



Comet assay, was used during our experiments for evaluation of DNA damage in erythrocytes of fishes and lizards and haemocytes of crayfishes



Olive Tail Moment definition



Tail DNA% = 100 x Tail DNA Intensity/Cell DNA Intensity

Tail Moment can be measured using one of the following methods:

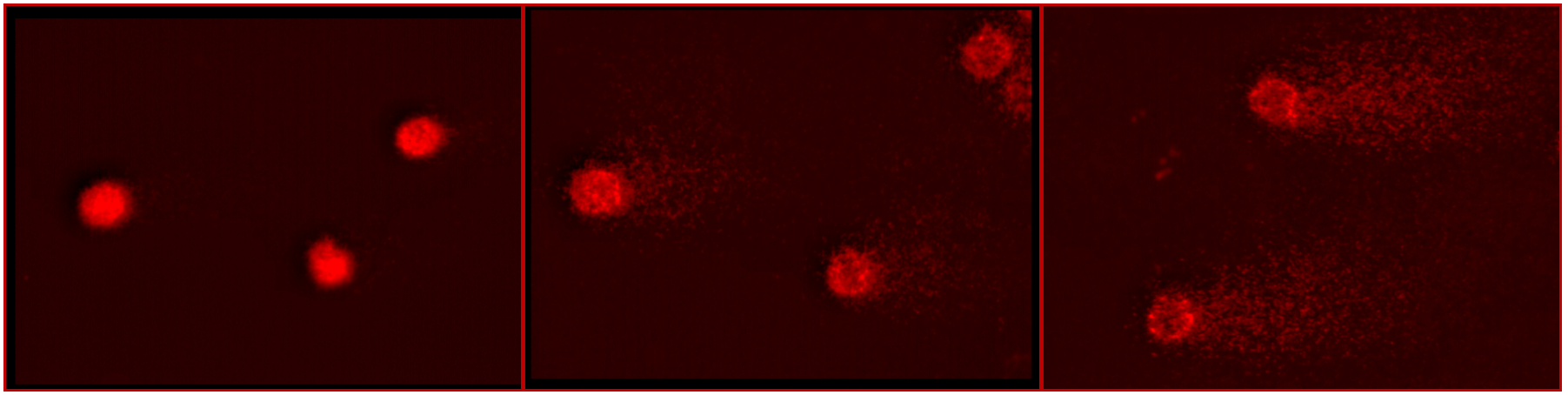
(a) Olive Tail Moment = Tail DNA% x Tail Moment Length*

(b) Extent Tail Moment = Tail DNA% x Length of Tail (see diagram on left)

A number of Comet analysis software programs are commercially available, such as LACAAS from Loats Associates, Inc.) and Comet Assay IV from Perceptive Instruments.

*Tail Moment Length is measured from the center of the head to the center of the tail (see diagram)

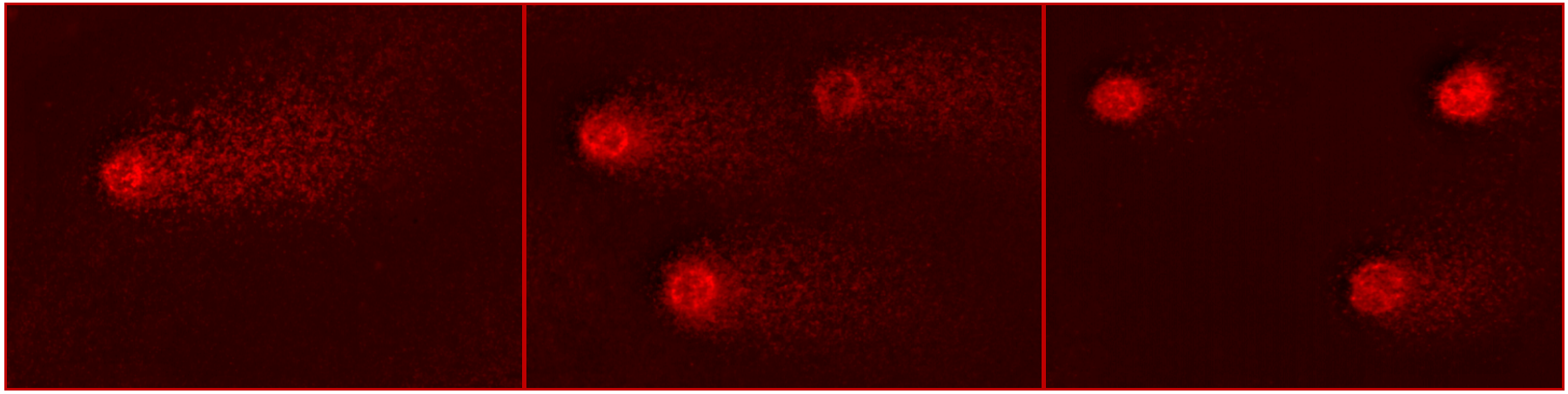
Comet assay images of K-562 cells after irradiation by AREAL accelerator



Non-irradiated control

Irradiation, dose 2 Gy,

Irradiation, dose 4 Gy,



Irradiation, dose 8 Gy,

Irradiation, dose 16 Gy,

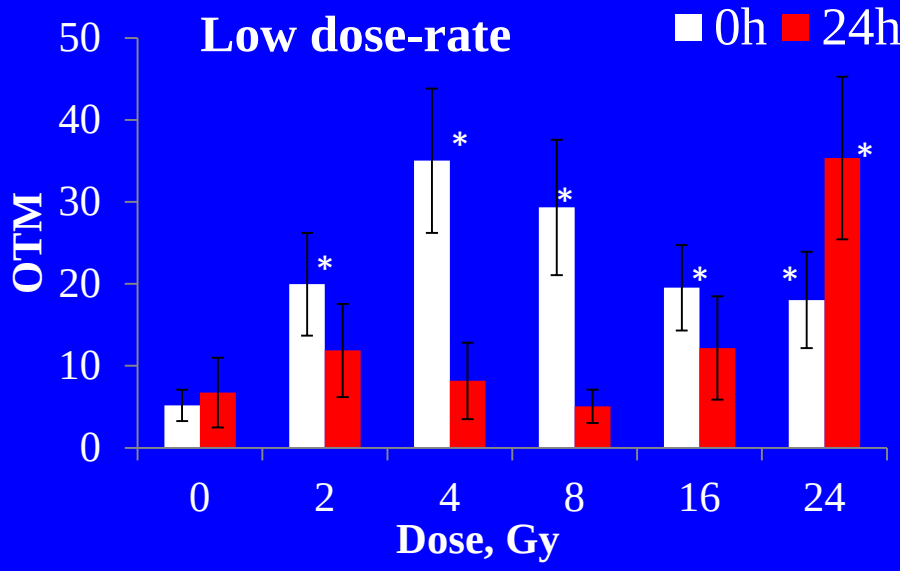
Irradiation, dose 24 Gy,

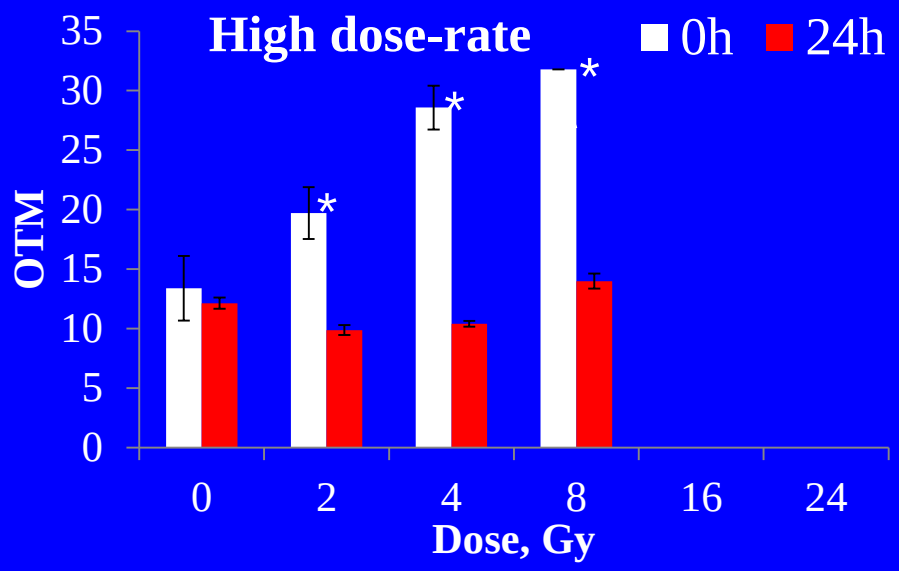
The exposure of K562 human chronic myelogenic leukaemia cells to AREAL ultrafast electron irradiation at different doses revealed the dose-dependent increase of the primary DNA damage.
The comet assay was performed immediately after the irradiation to avoid DNA repair.

At the doses higher than 4 Gy the DNA damage decreases. The reason - the level of dead cells can be increased in relation to viable cells. After 24 hour of cell incubation, the damaged DNAs have repaired until 24 Gy.

It was shown that electrons, as a source of low LET radiation, led to isolated DNA lesions, including single-strand and double-strand breaks of DNA, which were generally repaired efficiently.

DNA damage (Olive Tail Moment) in K-562 cells after 0 and 24 hours after irradiation (pulse frequency **2 Hz and 20 Hz**)





Low dose-rate – 3,6 Gy/min (**pulse frequency 2 Hz**), High dose-rate – 36 Gy/min (**pulse frequency 20 Hz**)

- The radiation-induced levels of DNA damage depend on dose and dose-rate

Distribution of cells on the base of DNA damage in K-562 cells after irradiation by low and high dose rate, by Olive Tail Moment

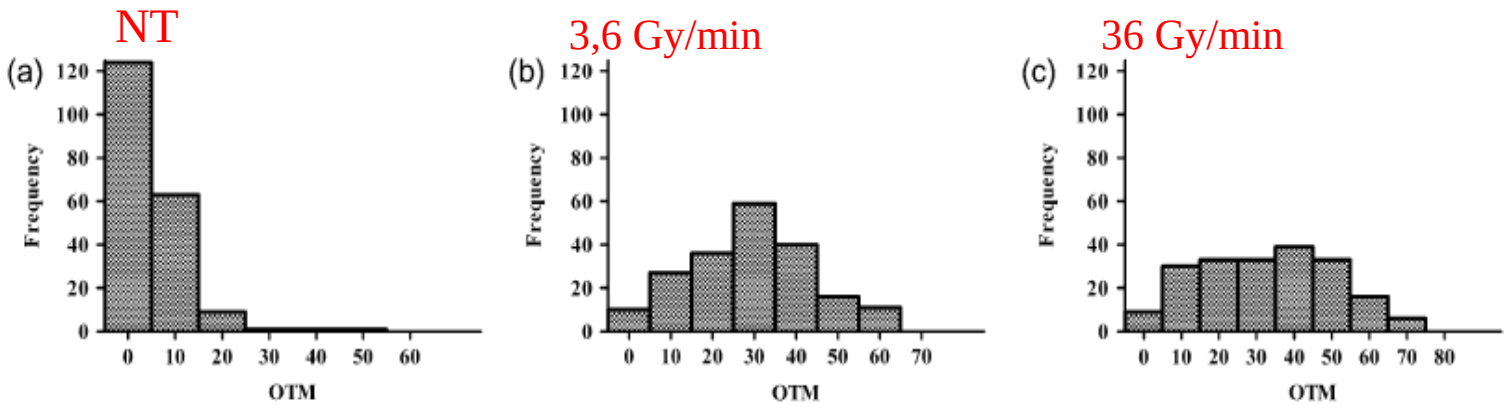


Fig. 1. Probability distribution of the OTM value for unirradiated K-562 cells (a) and those exposed to 8 Gy with the ultrashort electron beam at low (3.6 Gy/min) (b) and high (36 Gy/min) (c) dose rates at the 0 h time point after irradiation.

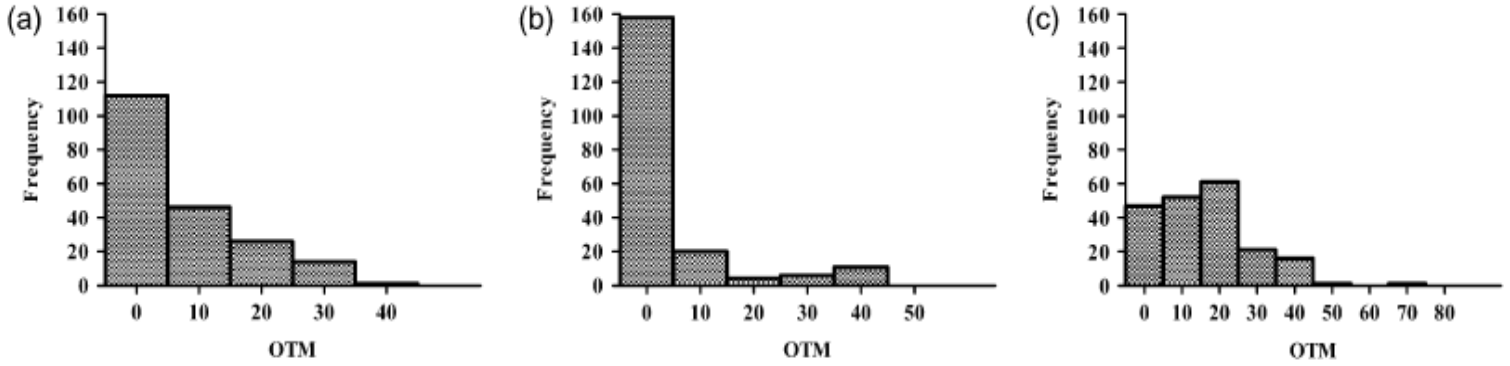


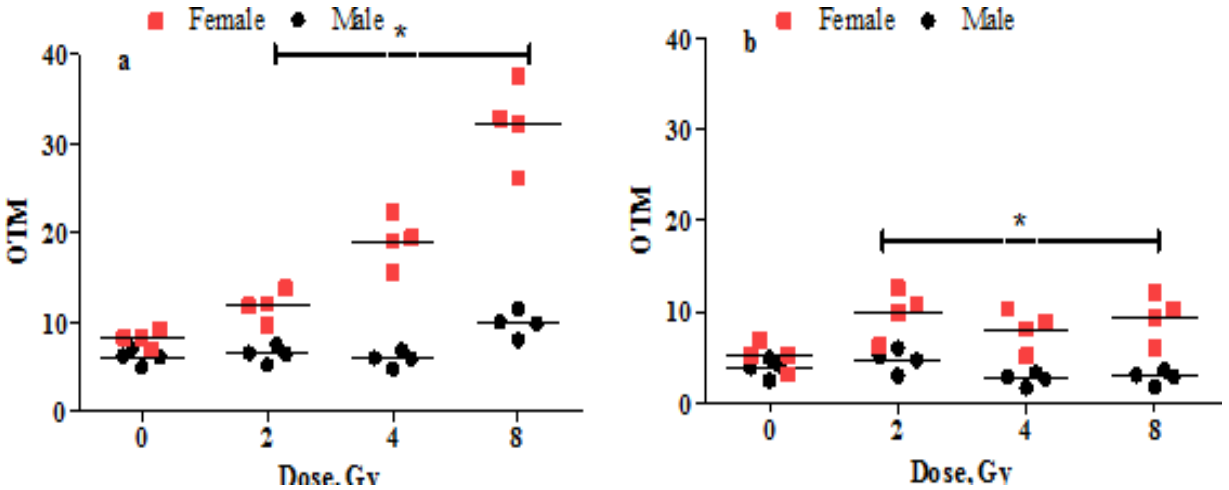
Fig. 2. Probability distribution of the OTM value for unirradiated K-562 cells (a) and those exposed to 8 Gy with the ultrashort electron beam at low (3.6 Gy/min) (b) and high (36 Gy/min) (c) dose rates at the 24 h time point after irradiation.

Gender-dependent radiosensitivity in clinical radiobiology **can have an impact on tumour response during radiation therapy, as well as on early and late side effects, including the induction of secondary malignancies.**

There are sporadic studies on the potential molecular and cellular bases for sex-specific radioreactions. Recently, the **significant differences in radiosensitivity, associated with genetic polymorphic variations, were shown between females and males** (Alsbeih G et al.,2016).

The recent report on mortality among atomic bomb survivors shows that **the excess relative risk of cancer is far higher for females than males**.

However, the biological and molecular bases of the sex-specific radioreactions are still need to be investigated (Preston DL et al.,2003).



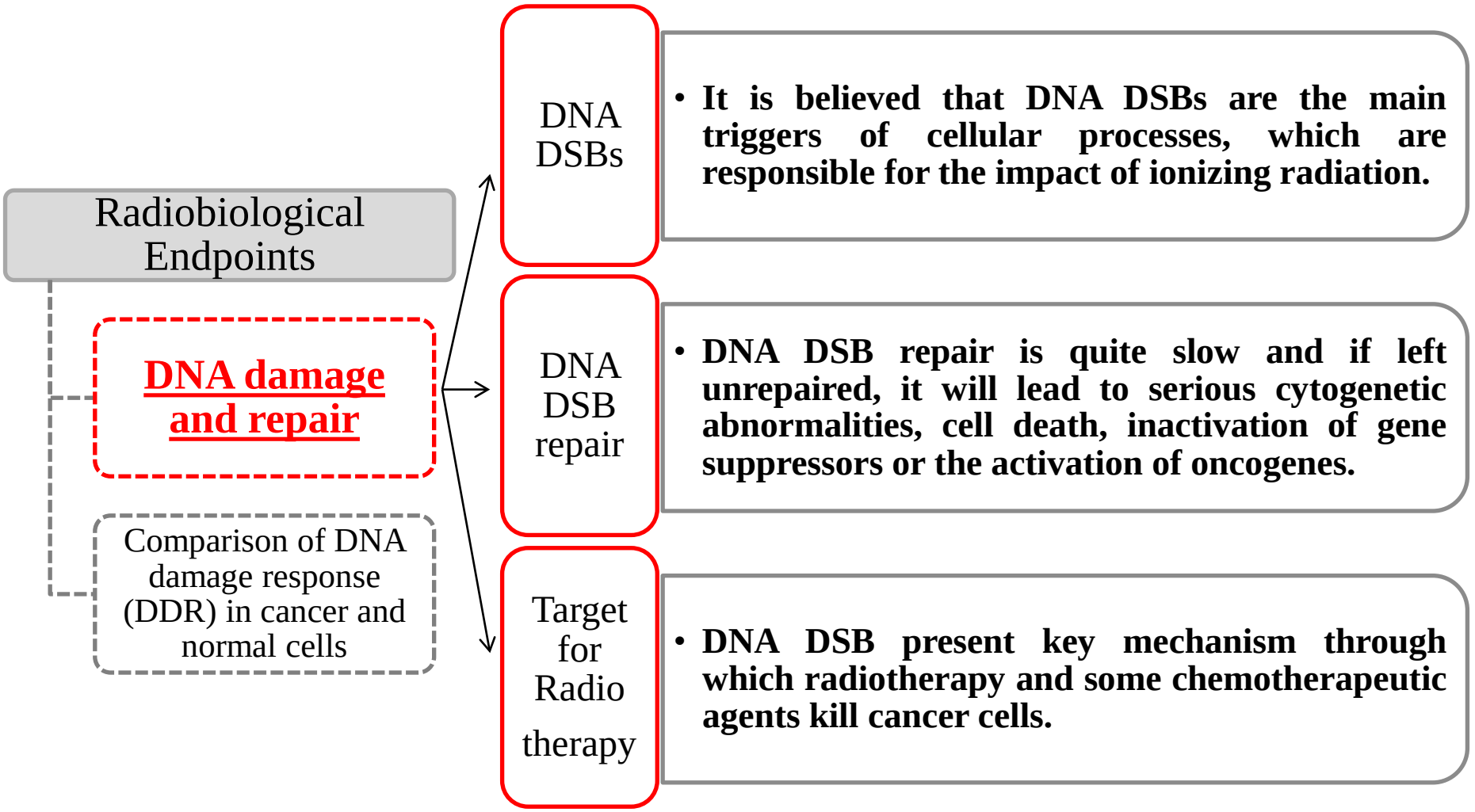
Two-way ANOVA analysis was performed to compare radiosensitivity of male and female groups.

The gender effect on radiation-induced DNA damage/repair was considered extremely significant after adjustment with Bonferroni correction ($p<0.0001$). **The female high radiosensitivity was demonstrated**, since the DNA damage level in female PBMCs increased more than **4 times** (8Gy) in comparison with control.

After 24h of incubation the **reduction of DNA-damage was evident**, but the overall level of DNA damage still was significantly higher than that of in control.

The level of DNA damages increased less than 1,5 times in comparison with control and those damages were repairable

Detection of DNA DSBs (damage and repair)



An immunocytochemical analysis of proteins participating in the processes of DNA DSBs (FOCI assay) was used

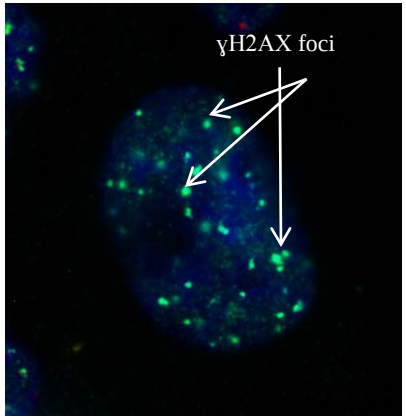
During recognition and repair of DSBs the **dynamic microstructures, called foci, are formed**. They contain hundreds to thousands of copies of various proteins involved in these processes (gamma-H2AX, phospho-ATM, 53BP1, RAD51 etc).

The quantitative analysis of the foci of repair proteins and their localization/colocalization in the postradiation period
allows to determine not only the number of DSBs and their spatial distribution in the cell nucleus, but also the efficiency and the mechanisms of their repair.

Detection of DNA DSBs formation and repair after ultrashort pulses of electron beam irradiation in human fibroblasts using immunohistochemistry.

DNA damage foci are distinct spots in the subnuclear region after DNA damage consisting of repair proteins of double-strand DNA ruptures

FOCI contain hundreds to thousands of copies of various proteins involved in DNA DSB repair

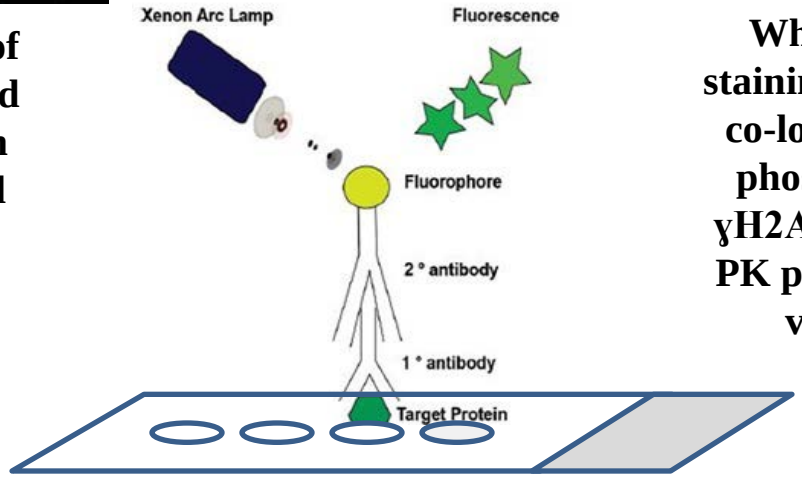


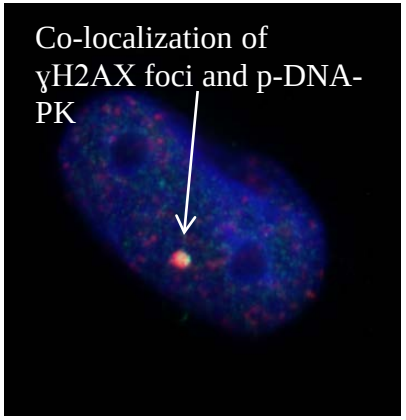
Visualization of phosphorylated γH2AX foci in irradiated cell

Fluorophores

γH2AX foci – Fluorescein (FITC) – green

p-DNA-PK - Texas Red - red





When double-staining is used, the co-localization of phosphorylated γH2AX and DNA-PK protein can be visualized

DNA DSBs recognition and repair (FOCI assay)

What is IRIF (irradiation induced foci)

- DNA damage foci are distinct spots in the subnuclear regions after DNA damage, consisting of repair proteins of double-strand DNA ruptures

What is γH2AX foci (DNA DSBs recognition)

- **One of the earliest (several minutes) of DNA repair events is the phosphorylation of a protein called histone H2AX**

• H2AX is a variant of histone H2A, which is a core component of nucleosome structures around which DNA is wrapped

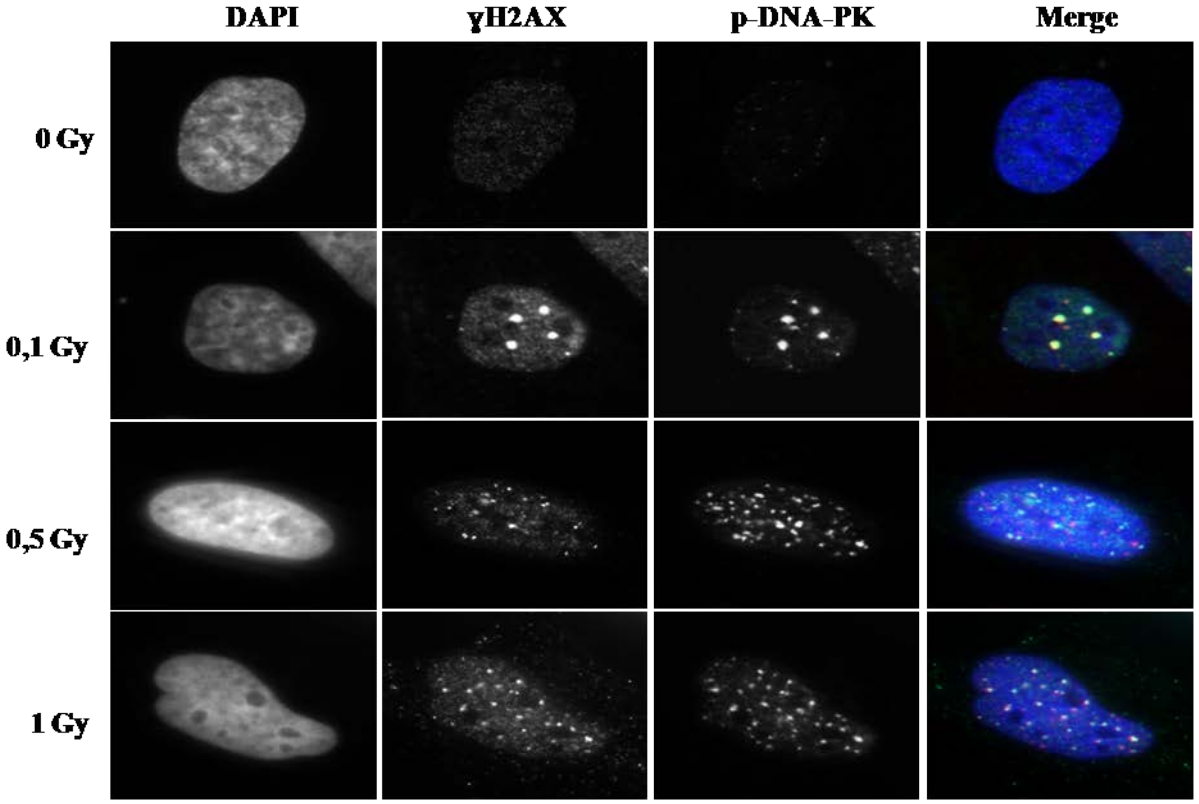
- **Phosphorylated protein, which is designated as γH2AX, is required to involve many other proteins in the repair process.**
- **Phosphorylation of H2AX occurs with the participation of kinases, which are sensors of double-strand breaks of DNA *(For low-LET reasonable are* DNA-PK and ATM*)***

Other Foci (DNA DSBs repair proteins)

- **NHEJ (non-homologous end-joining) and HRR (homologous recombination repair) are the main DNA DSBs repair pathways**
- **Detection of p-DNA-PK foci can serve as a marker of NHEJ pathway activation**
- **Detection of MRE11 foci can serve as a marker of HRR pathway activation**

Co-localization of γH2AX foci and p-DNA-PK

The ultrashort electron beam radiation effect on DNA DSBs formation and repair



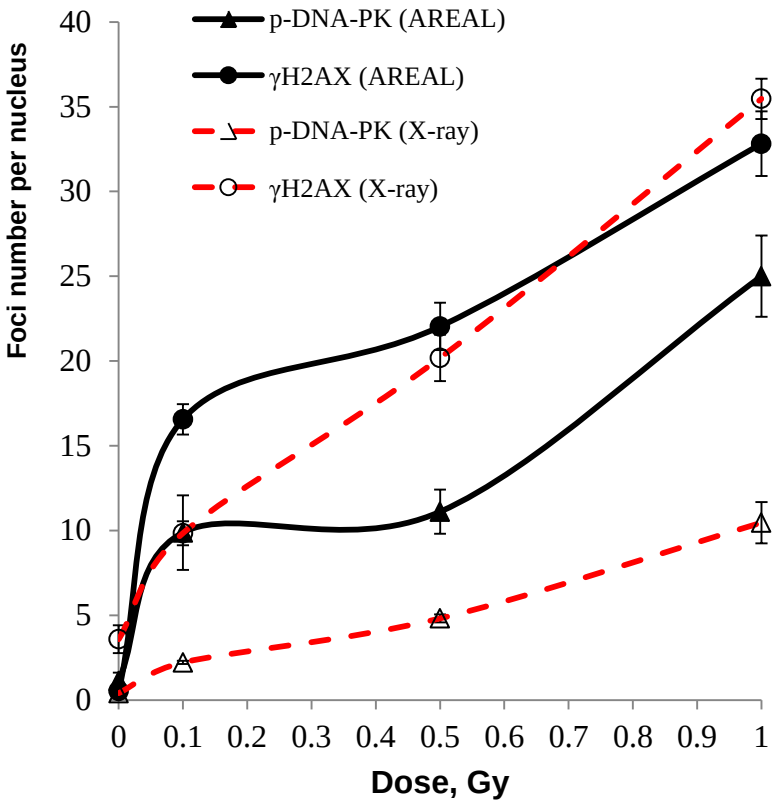
The γH2AX and p-DNA-PK foci and their co-localization at 1h post-irradiation in human fibroblasts

The dose-dependant increase and time-dependant decrease of γH2AX and p-DNA-PK foci was observed after ultrashort pulsed electron beam and X-ray irradiation (reference irradiation).

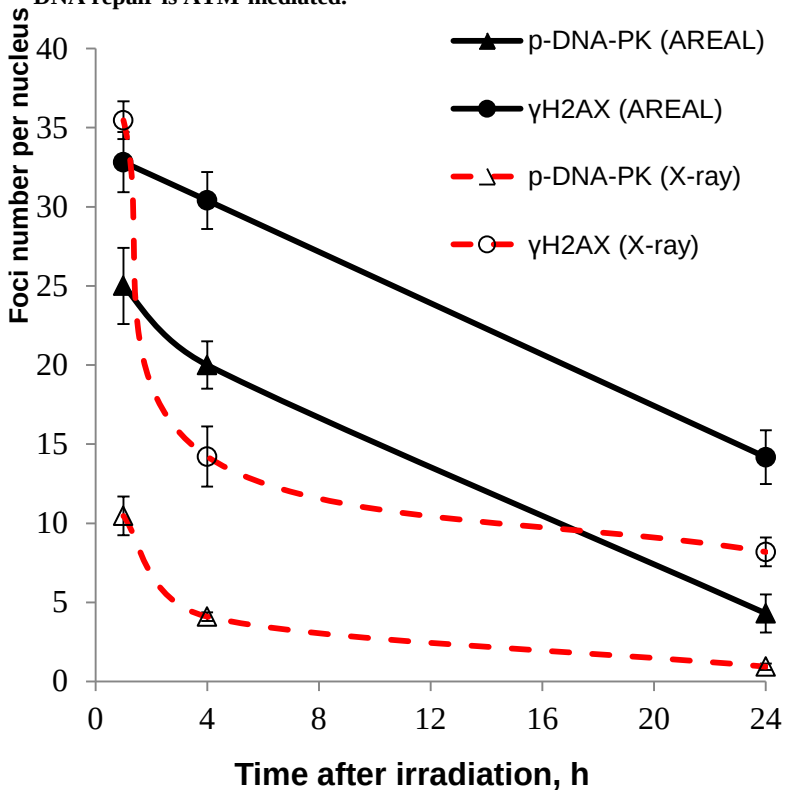
Since, the NHEJ is a dominant pathway in DSB repair induced by low-LET radiation (such as X- and γ-rays or electron beam radiation), we analyzed the expression of p-DNA-PK as a key protein in the NHEJ pathway.

Dose-effect curve –

The yield of γH2AX foci was similar for AREAL and X-ray , whereas the expression of p-DNA-PK was lower in case of X-ray .



1.In case of electron beam irradiation at the dose of 1 Gy, the high level of non-repaired residual γH2AX foci (up to 15 foci per cell) was observed,
2.After 24 h. we see effective repair for X-ray,
indicating the formation of complex DNA damages with delayed repair.
3.In case of electron beam irradiation at the dose of 1 Gy
the repair of 80% of DSBs was mediated by p-DNA-PK.
4.In case of X-ray irradiation, activity of p-DNA-PK was lower, perhaps the DNA repair is ATM-mediated.



So, electron beam irradiation characterized by slow repair kinetics induces more complex DNA damages in comparison to X-ray

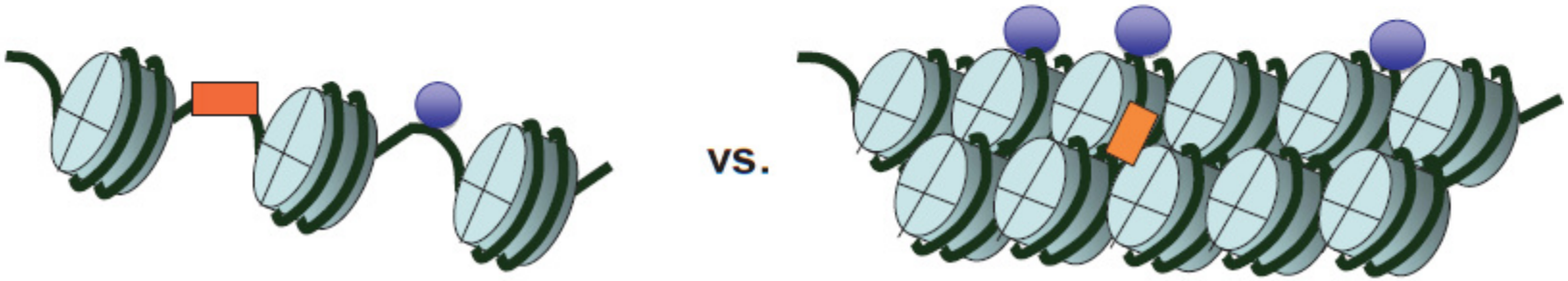
The differences in p-DNA-PK activity provide evidence about difference in DNA damage response after two types of irradiation.

EPIGENETICS describes heritable changes in gene function that cannot be explained by changes in DNA sequence

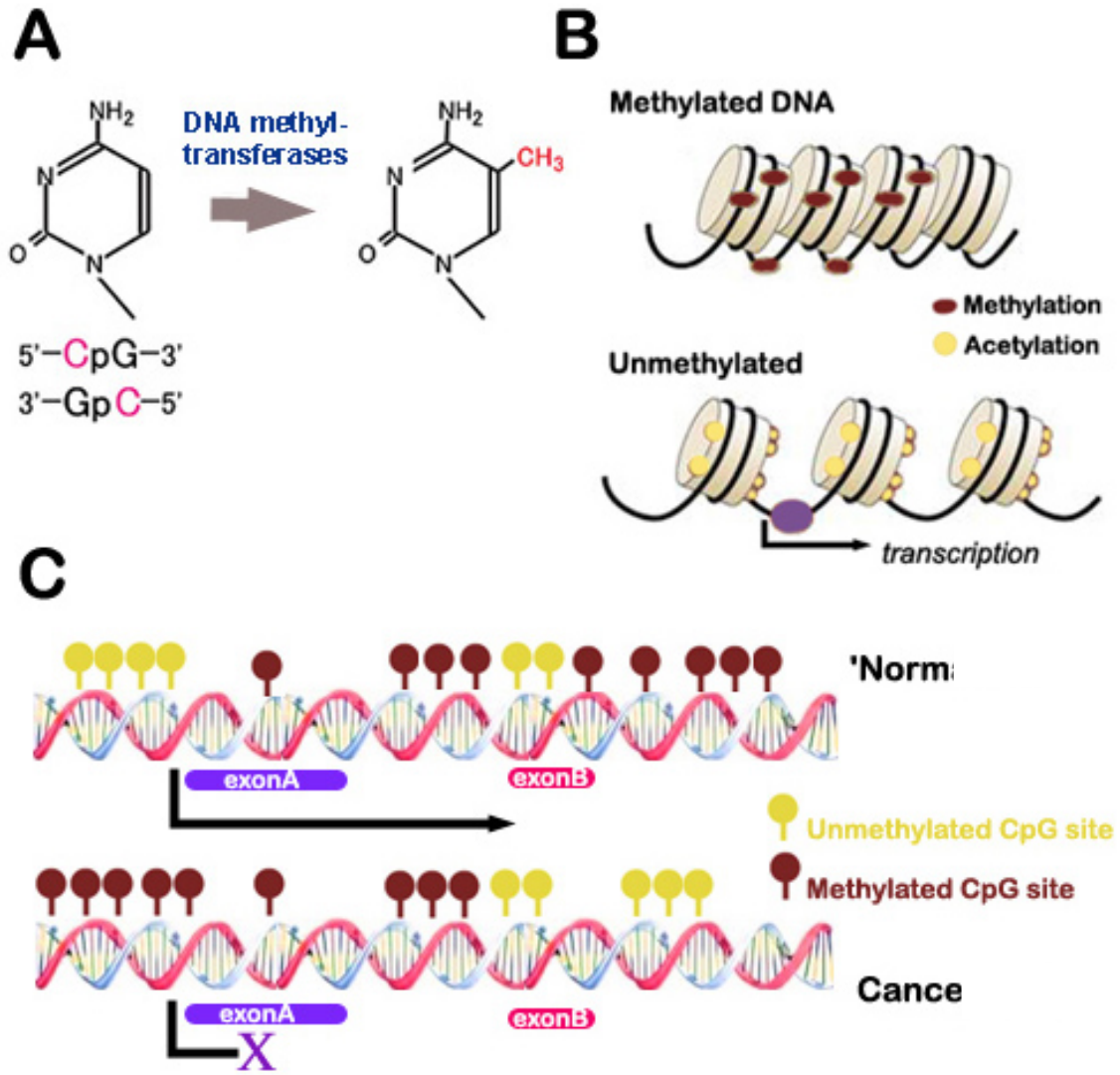
Epigenetic modification alters which genes are on or off

ON

OFF



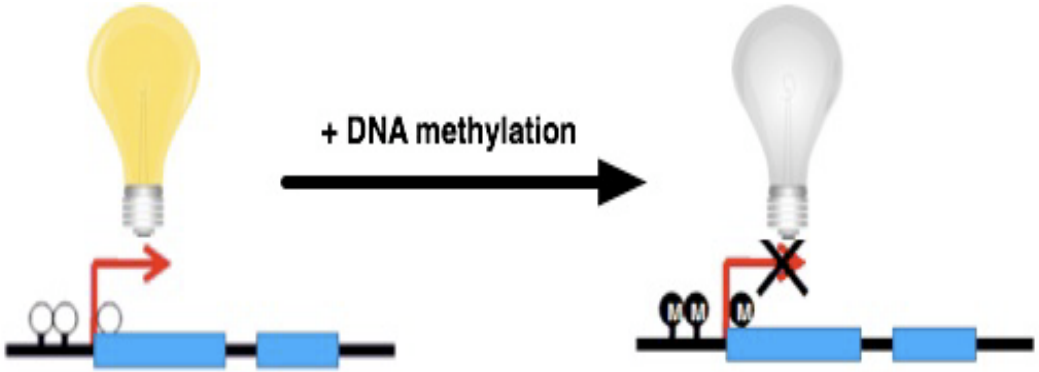
DNA methylation



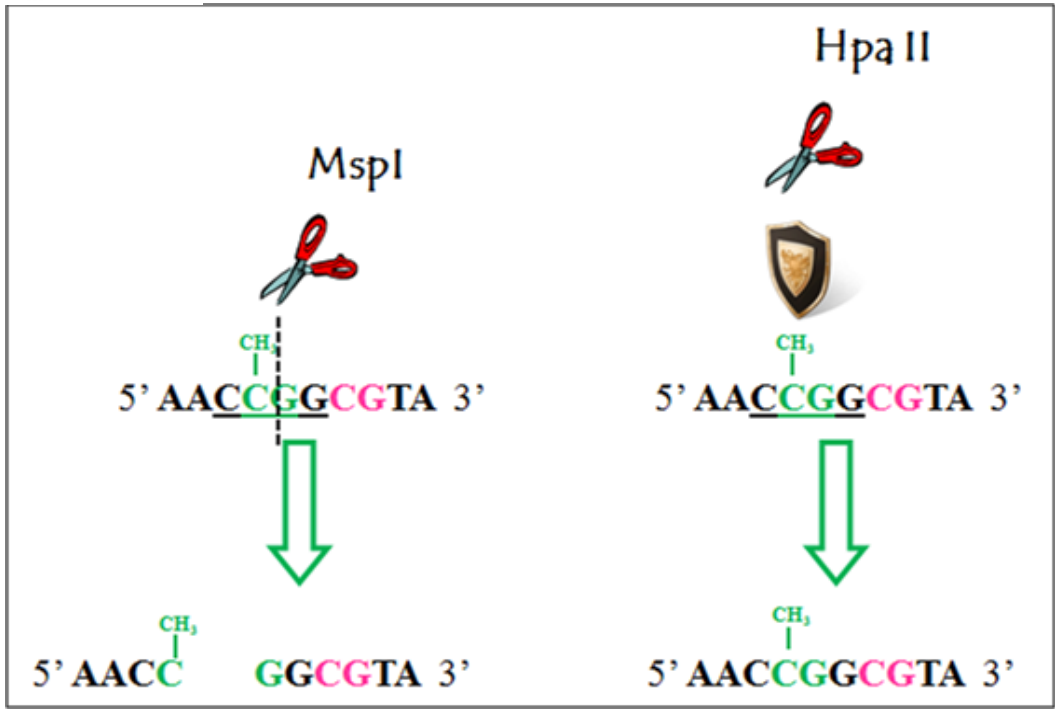
Hypomethylation

Hypermethylation

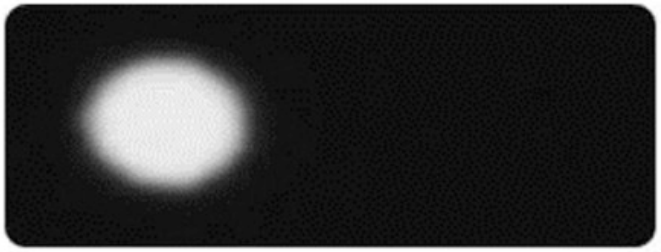
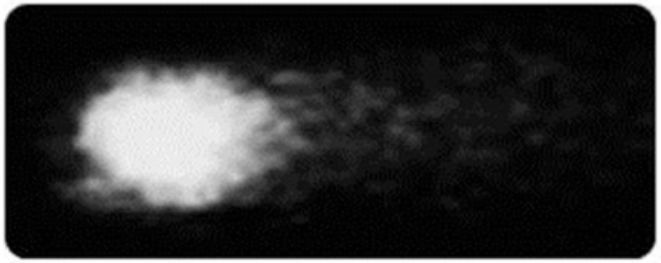
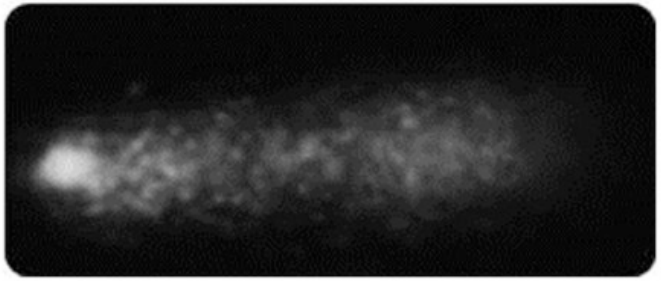
DNA methylation



Silencing by promoter methylation



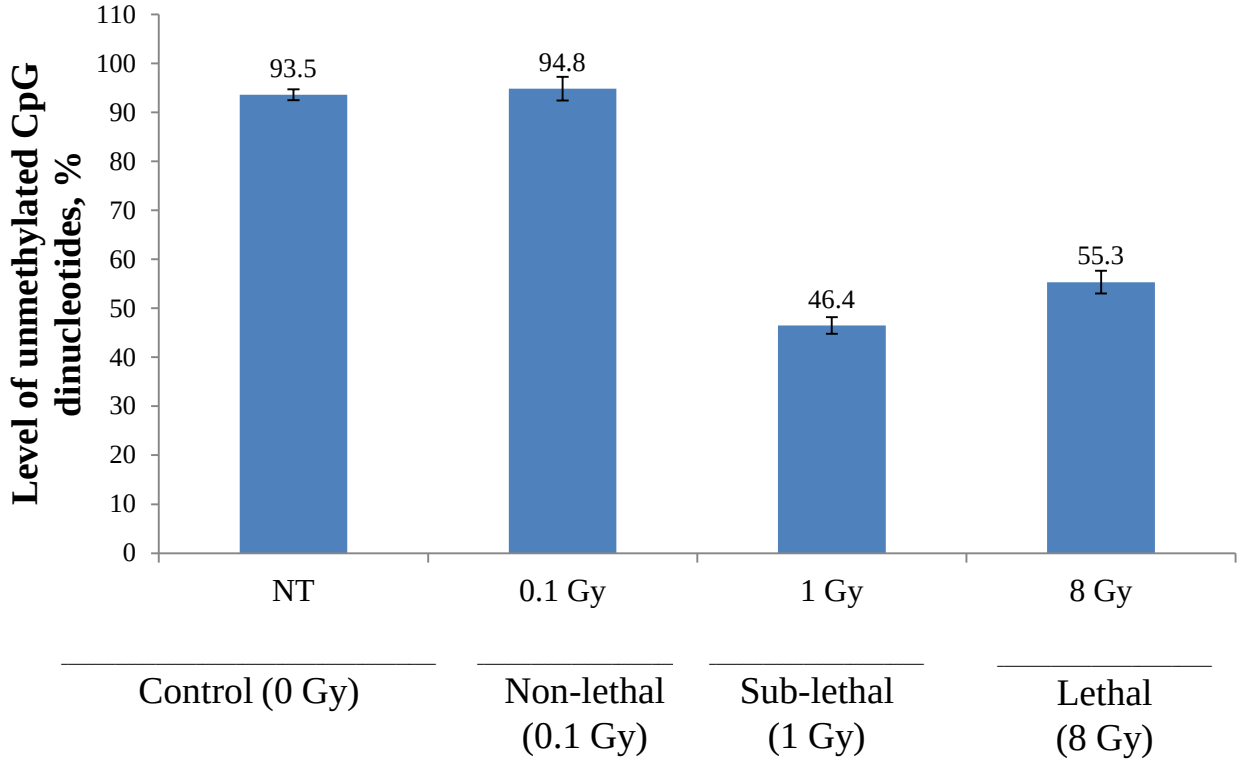
Application of Comet assay comet assay to evaluate the global DNA methylation

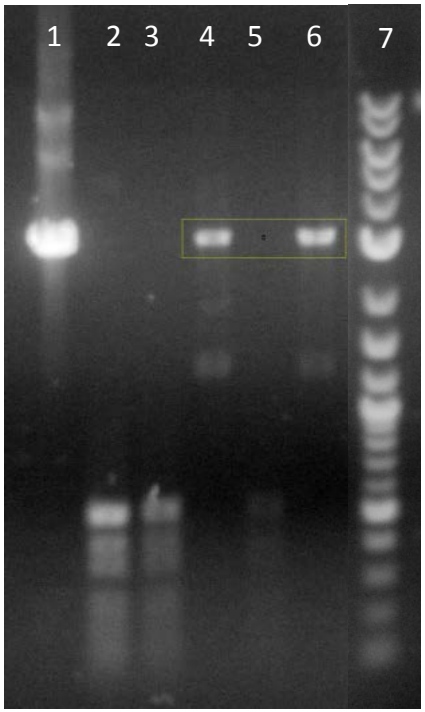
Control cells HepG2	
HepG2 cells treated by HpaII	
HepG2 cells treated by MspI	

Lewies et al. Using a medium-throughput comet assay to evaluate the global DNA methylation status of single cells. Front Genet. 2014; 5:215.

The level of global DNA methylation after ultrashort pulsed electron beam irradiation

EpiJET DNA Methylation Analysis Kit (MspI/HpaII) uses the MspI and HpaII restriction enzymes to analyze DNA methylation status





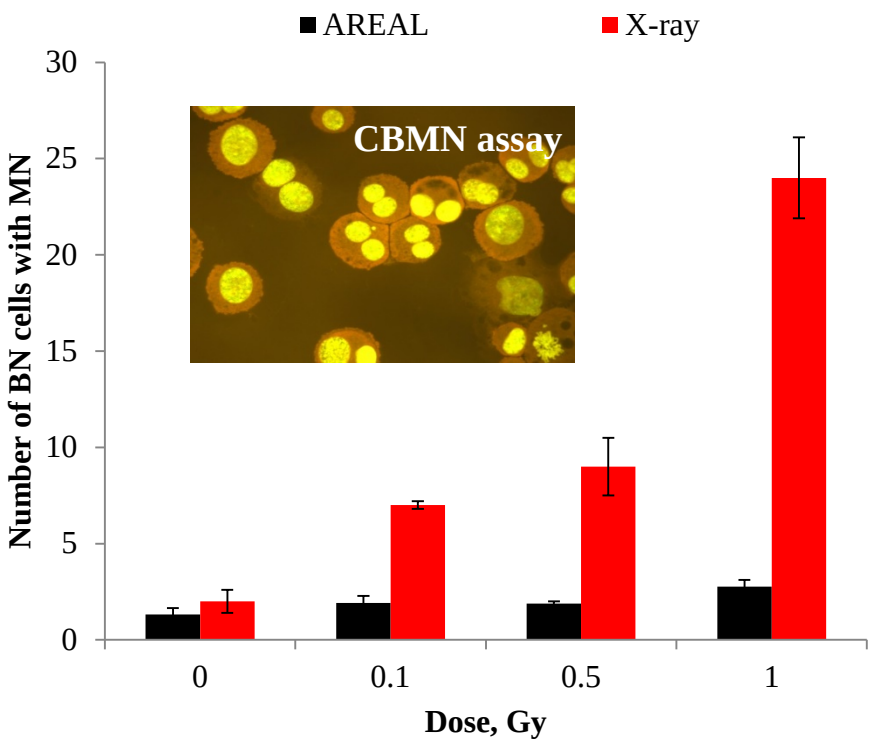
1 –Unmethylated DNA (UD)
2 - UD+Msp
3 - UD+Hpa
4 – Methylated DNA (MD)
5 – MD+Msp
6 - MD+Hpa
7 - Ladder

•The UPEB radiation induces changes in global DNA methylation 24 h post-irradiation at the sub-lethal (0.1 Gy) and lethal (8 Gy) doses of irradiation. The level of methylated CpG islands increased by 40-50%.

•At the non-lethal (0.1 Gy) dose of irradiation no changes in the status of global DNA methylation was observed

•The observed changes in global DNA methylation status may influence the expression profile of DNA damage repair genes

The induction of chromosomal aberrations, such as chromosomal fragments resulting from DNA breaks, or entire chromosome loss was evaluated in MRC5 cell line after 0.1 Gy, 0.5 Gy and 1 Gy doses of electrons and X-ray irradiation.



•The slight, but statistically significant increase was observed only at the **1 Gy of electrons** irradiation

•The dose-dependent increase of MN frequency was observed after X-ray irradiation, reaching the level of 24±2.1 of BN cells with MN at the irradiation dose of 1 Gy

To compare the mutagenic capacity of the UPEB with X-ray irradiation, the RBE (relative biological effectiveness) value was calculated as the ratio of the **slopes** for the two radiation types

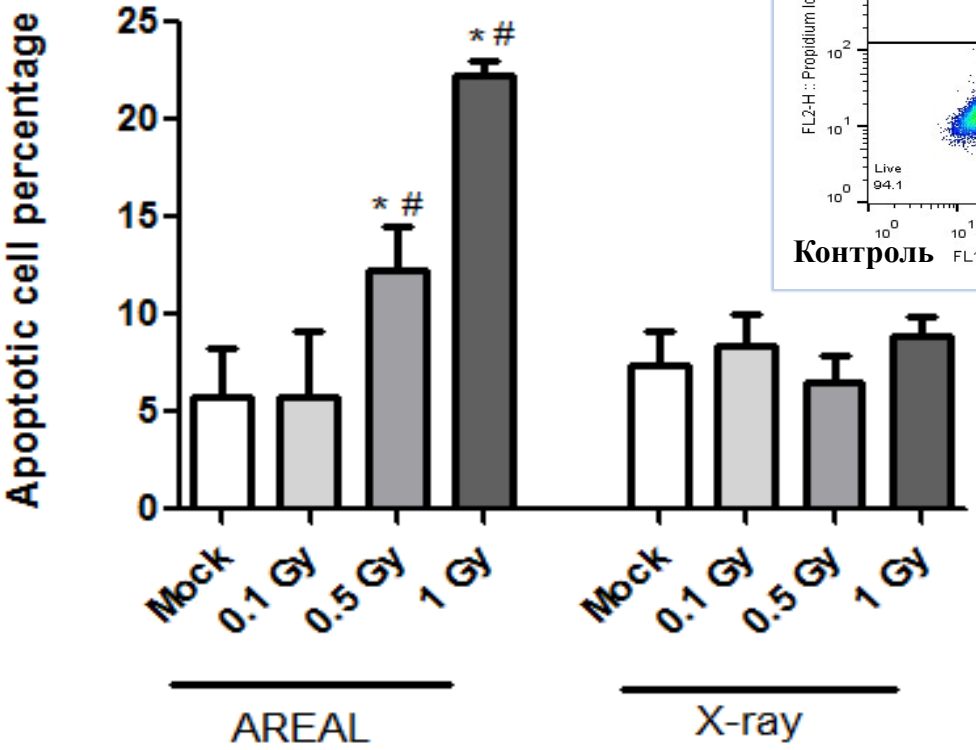
The dose-response functions were

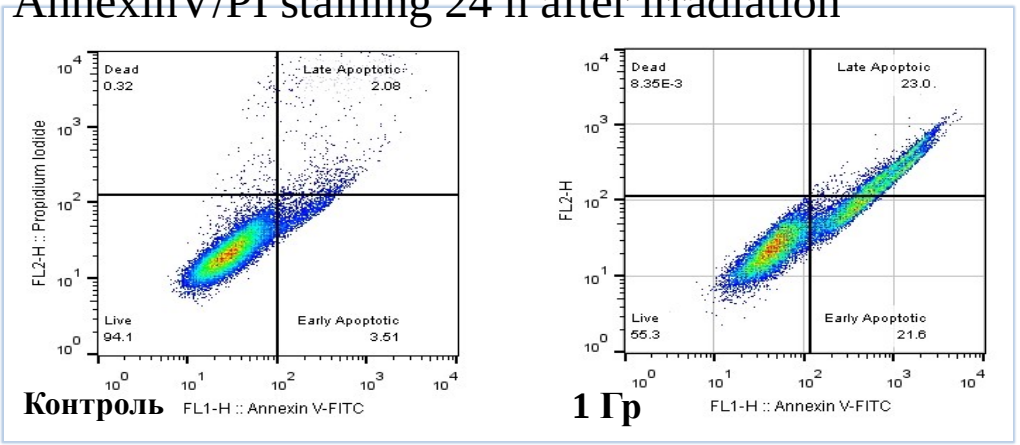
•AREAL $y=\mathbf{1.1979}x+1.4864$ ($R^2=0.83$)

•X-ray $y=\mathbf{20}x+2.5$ ($R^2 = 0.92$)

The deduced RBE value of UPEB radiation to X-rays to induce micronuclei in MRC5 cells was about 0.06, suggesting **lower level of mutagenic capacity in case of electrons iradiation**

The viability of MRC5 cells, as well as the incidence of apoptotic cell death induced by 0.1 Gy, 0.5 Gy and 1 Gy doses of UPEB and X-ray radiation was evaluated using flow cytometric analysis by AnnexinV/PI staining 24 h after irradiation





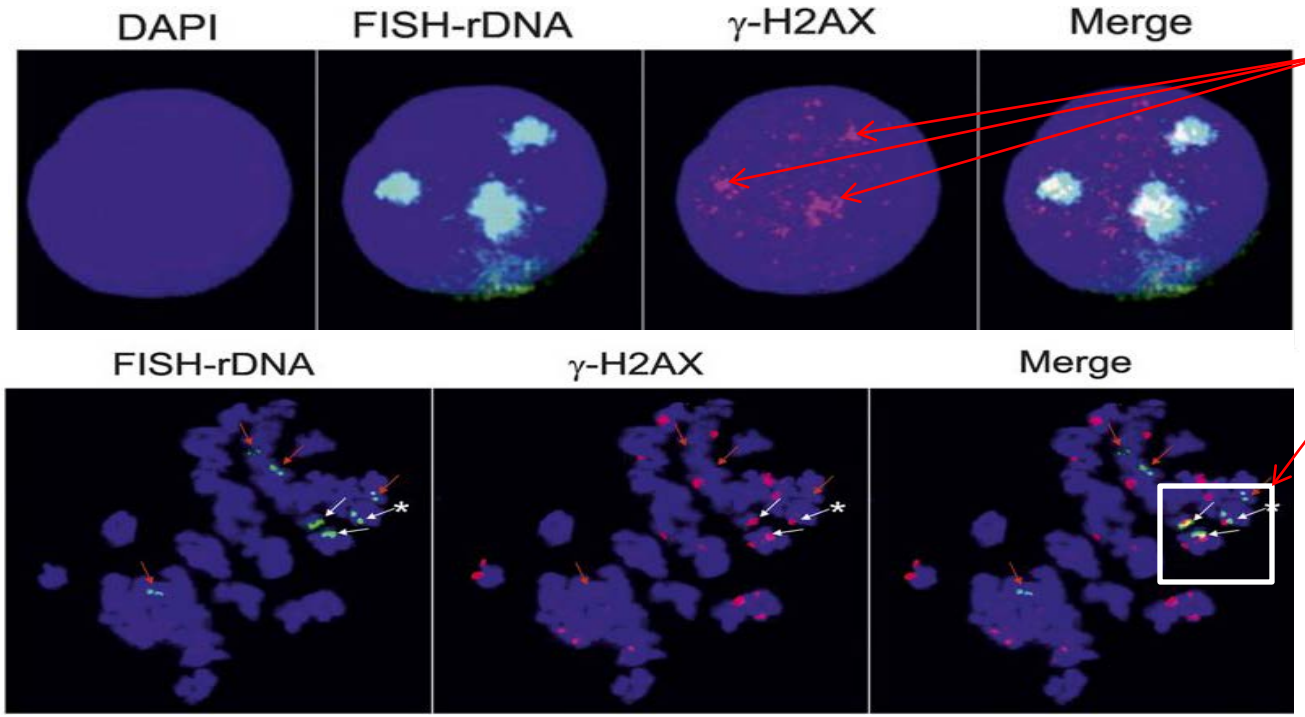
!!! The low level of genomic abnormalities passing through generations of cells (CBMN assay) accompanied with faster elimination of damaged cells is of great interest for improvement of radiotherapy efficiency associated with reduction of early and late side effects of exposure.

- As it was expected, no significant changes in cell viability were found at all doses of X-ray irradiated cells after 24h
- 0.5 Gy and 1 Gy doses of UPEB irradiation resulted in significant increase in percentage of apoptotic cells at 24 h post-irradiation
- At the 1 Gy of irradiation the 60±3.4 % of viability was accompanied with the 22±1.4 % of apoptotic cells
- The increase of the level of apoptotic cells was observed without changes **in the population of primary necrotic cells, whereas the rest 20% of dead cell population was detected in late apoptotic/secondary necrosis quadrant of the dot plot**

Future direction: Combination of FOCI-FISH

can bring to detection by FISH of transcriptional hot spots where are formed DNA double-strand breaks

On rDNA, genes are continuously transcribed, due to ribosome synthesis. Foci can arise in actively expressed genes.



The lymphoblastoid cell line in interphase and metaphase was subjected to FISH and combined immunostaining to show the localization of rDNA and γ-H2AX marks

Tchurikov et al., Scientific Reports 6; 25866 (2016)

- The **largest γ-H2AX foci** are located mainly inside **central areas of the nucleoli.**
- The rest of the γ-H2AX foci are scattered inside the nucleus.

- **From 7 rDNA clusters (FISH - double green dots) only three clusters with γ-H2AX foci** are co-localized

- **So, only part of the rDNA clusters active during interphase in the previous cell cycle, possessing transcriptional hot spots of DSBs and γ-H2AX marks, were inherited in metaphase chromosomes**

New dimensions of molecular radiobiology

The application of combined FOCI-FISH method can help to elucidate the hot spots of endogenous and/or exogenous DNA DSBs formation.

It can also help to reveal correlations between DNA DSBs formation and transcription patterns.

CNV

<u>CNV – DNA copy number variation.</u> Sections of the genome are repeated.

CNVs are sequences of DNA of 50 bp or larger compared with its reference genome, 9.5% of the genome contributes to spontaneous CNVs.

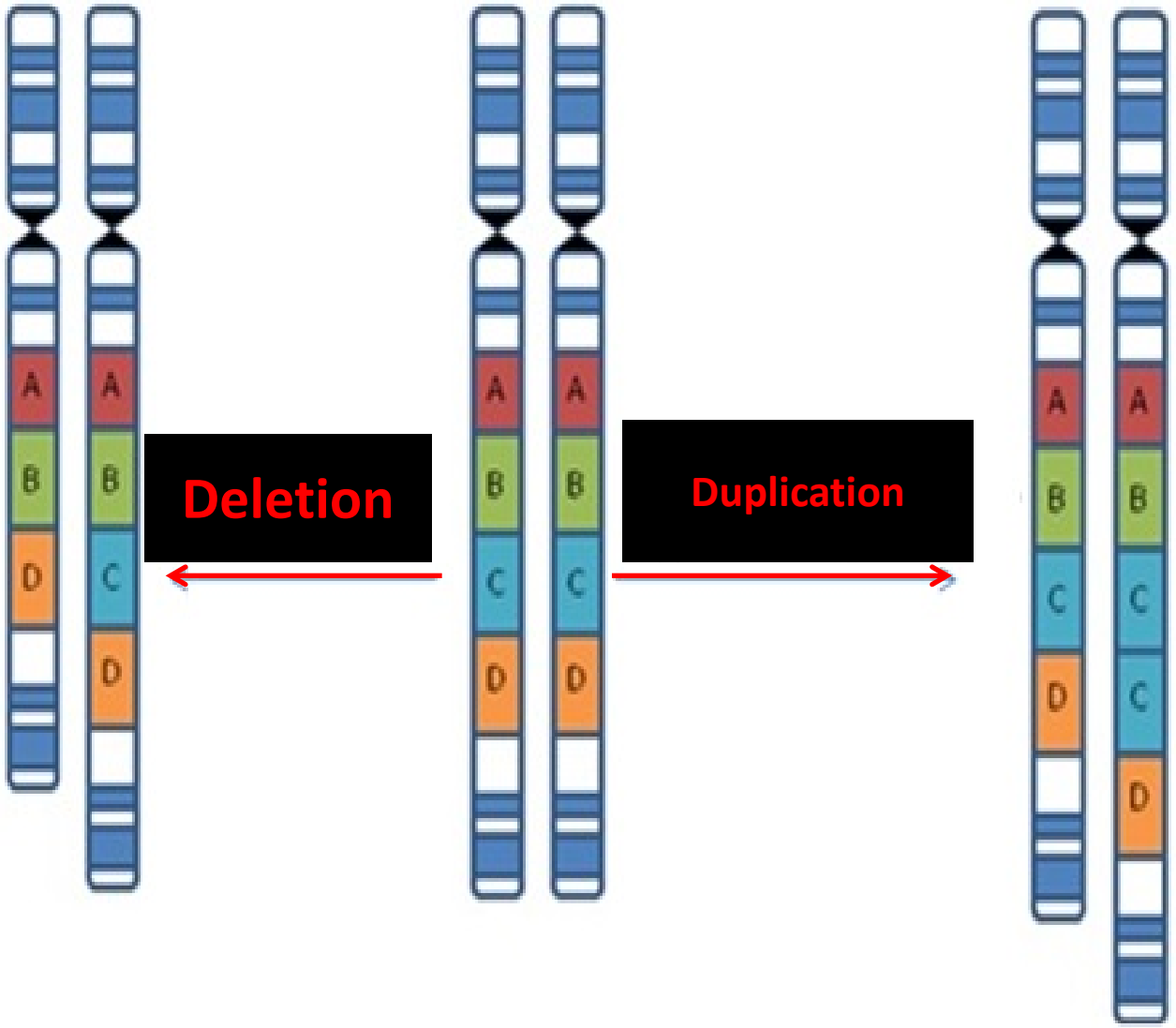
- **Copy number variants (CNVs) are important component of genetic diversity comprised of single nucleotide polymorphisms (SNPs), small insertion and deletion variants**, short tandem repeat polymorphisms, retrotransposable element insertion variants (e.g., Alu-s), inversion variants).
- *CNVs provide the raw material for gene family expansion and diversification, which is an important evolutionary force. CNVs can influence gene transcriptional and translational levels and have been associated with complex disease susceptibility.*

- **Copy number variation is now recognized as one of the major sources of genetic variation among individuals in natural populations of any species. Despite their importance little is known about environmental risk factors affecting CNVs.**

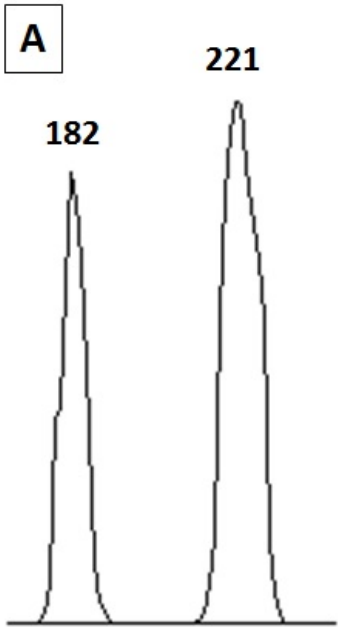
- **CNVs are mutagenic!(duplicated sequences can predispose to new mutation events**, like inversions, deletions and duplications)

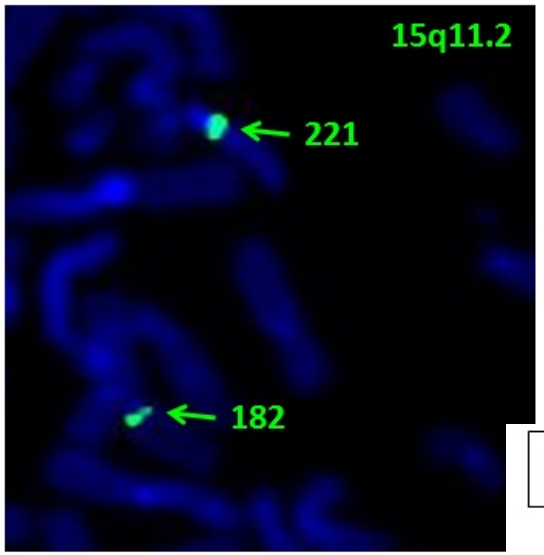
- **De novo CNVs can arise after influence of replication inhibitors (aphidicolin, hydroxyurea, ionizing radiation), these findings have been confirmed by our group. We studied induced CNVs with application of chemical and physical mutagens.**

Copy number variation - CNV



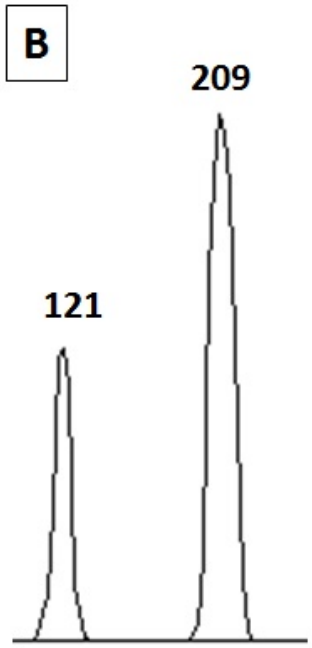
Analysis of fluorescence intensities using Scion Image - different sizes of probes signals

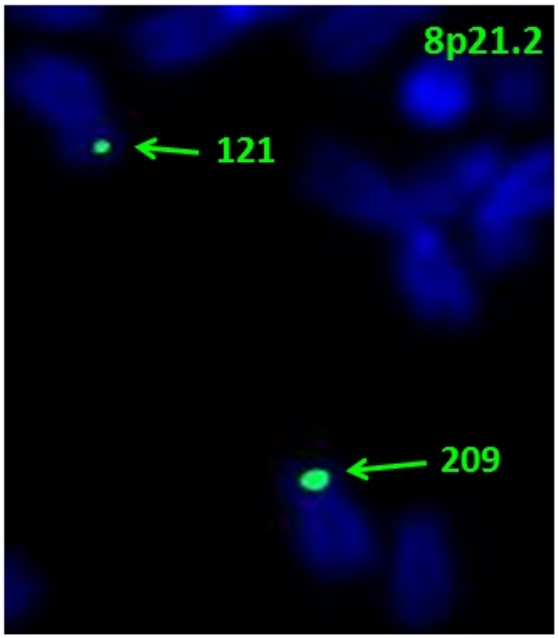




Fluorescence intensities of CNVs on homologous chromosomes 15q11.2

Fluorescence intensities of CNVs on homologous chromosomes 8p21.2





Harutyunyan *et al. Molecular Cytogenetics* (2015) 8:25
DOI 10.1186/s13039-015-0131-x

MOLECULAR CYTOGENETICS

RESEARCH Open Access

Influence of aflatoxin B1 on copy number variants in human leukocytes in vitro

Tigran Harutyunyan[1], Galina Hovhannisyan[1], Nelly Babayan[1,2], Moneeb AK Othman[3], Thomas Liehr[3] and Rouben Aroutiounian[1*]

Abstract

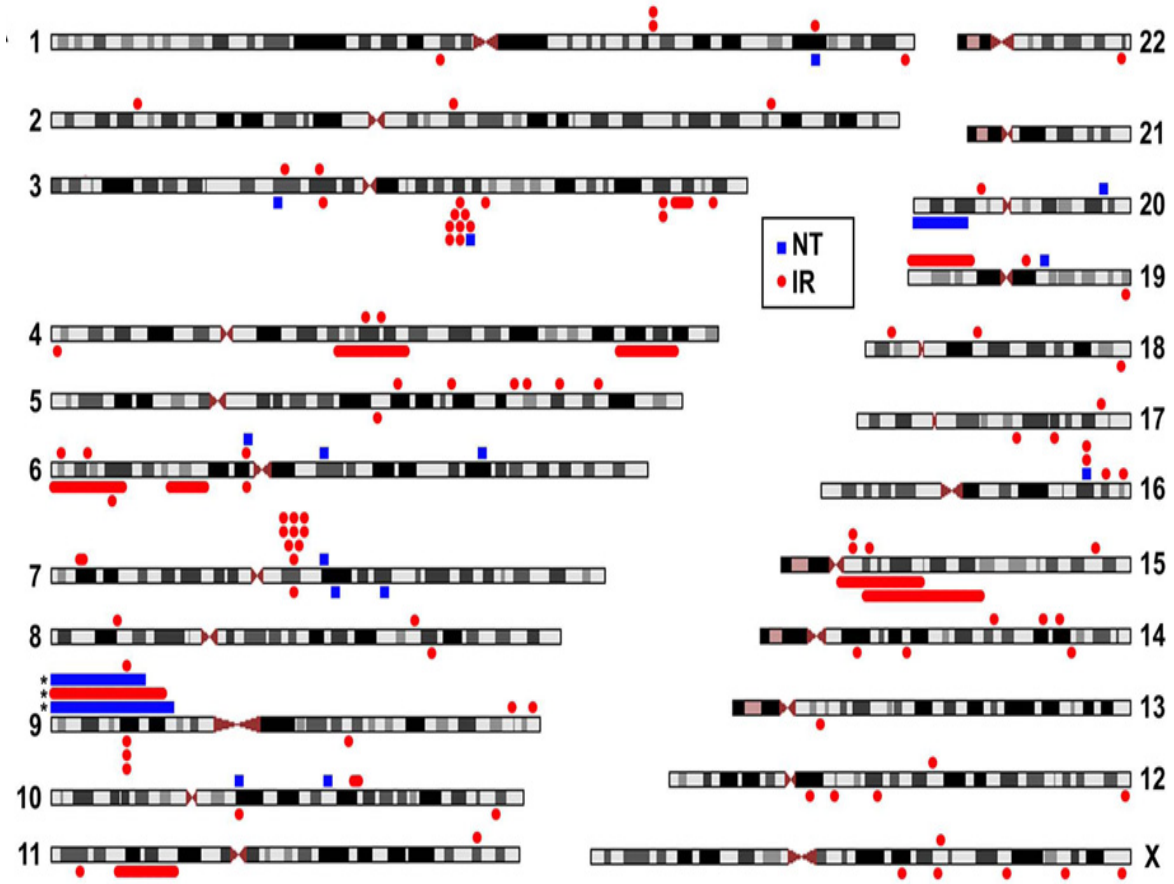
Background: Aflatoxin B1 (AFB1) is a mycotoxin produced by *Aspergillus spec.* The latter are worldwide contaminants of food with mutagenic and carcinogenic activities in animals and humans. AFB1 was shown to have deleterious effects on metabolism of eukaryotes in many model systems, including the ability to inhibit DNA replication. An agent that disturbs DNA replication may also have the potential to induce de novo DNA copy number variations (CNVs).

Results: Blood samples of three clinically healthy carriers were treated in vitro with AFB1 and chromosome preparations were subjected to parental origin determination fluorescence *in situ* hybridization (pod-FISH). Probes able to visualize CNVs in 8p21.2 and 15q11.2 were applied. In this setting here for the first time an influence of AFB1 on molecular-cytogenetically detectable CNVs could be shown.

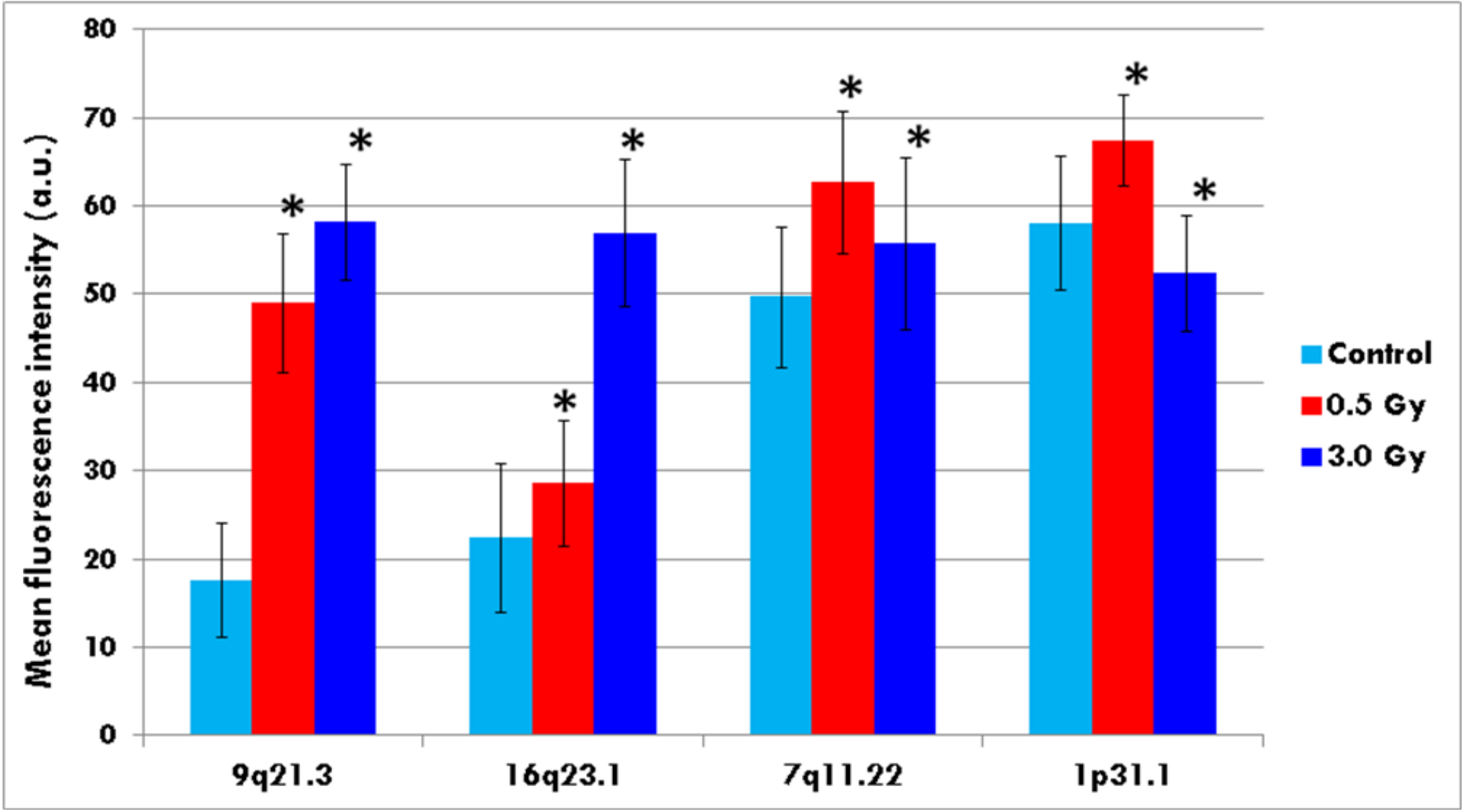
Conclusions: The obtained results indicate that: (i) pod-FISH is a single cell directed, sensitive and suitable method for the analysis of mutagen induced CNVs, (ii) AFB1 has the potential to induce *in vitro* instability of known CNVs in human leukocytes.

Keywords: Aflatoxin B1, Mycotoxins, Copy number variation, Parental origin determination fluorescence *in situ* hybridization (pod-FISH)

Ionizing radiation (1.5-3 Gy) induces copy number variations (CNVs) of DNA sites.



Arlt et al. Copy number variants are produced in response to low-dose ionizing radiation in cultured cells. Environ Mol Mutagen. 2014; 55(2):103-13. Irradiation by Philips RT250 (Kimtron Medical)



- **Mean fluorescence intensities of BAC signals at CNV loci** measured by ImageJ program.
- Irradiation with 3 MeV, 2 Gy/min, 2 Hz
- Ultrashort electron pulses at a dose of 0.5 Gy induced duplications at 9q21.3, 16q23.1, 7q11.22 and 1p31.1.
- At the dose of 3 Gy irradiation induced duplications at 9q21.3, 16q23.1 and 7q11.22, and deletion at 1p31.1.
- *-$p<0.05$ compared to control.

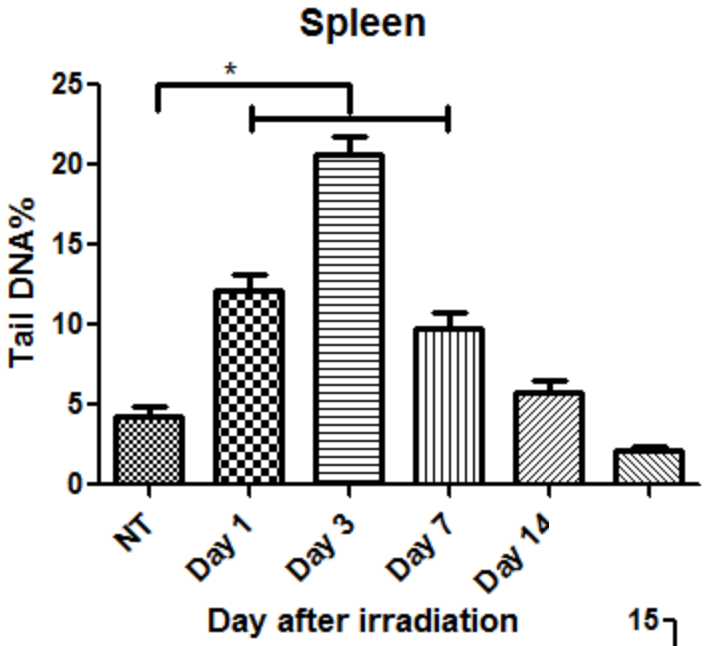
Experiments in vivo

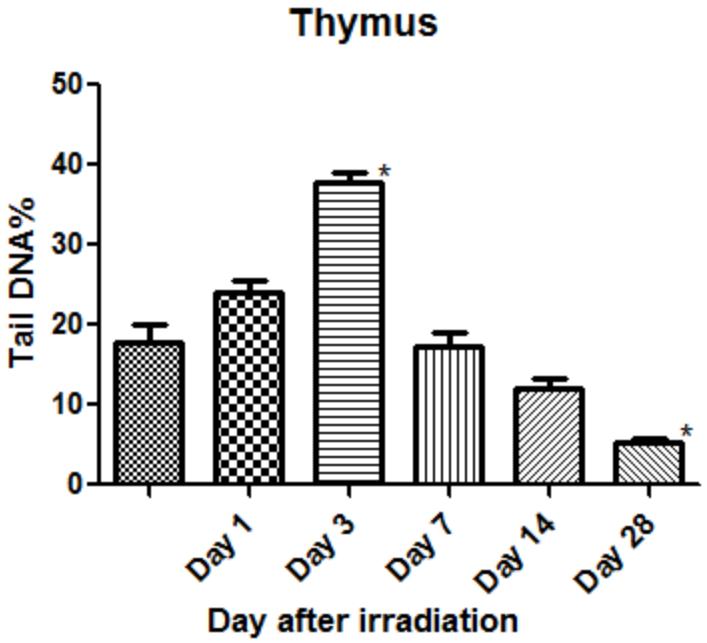
Since the cells of the hematopoietic system are the most sensitive to ionizing radiation, we investigated the effects of electron radiation on the **bone marrow, thymus and** spleen of white rats 160-170 g, males, irradiated at a dose of 2 Gy (pulse frequency 2 Hz), which is the dose of LD50 / 30.

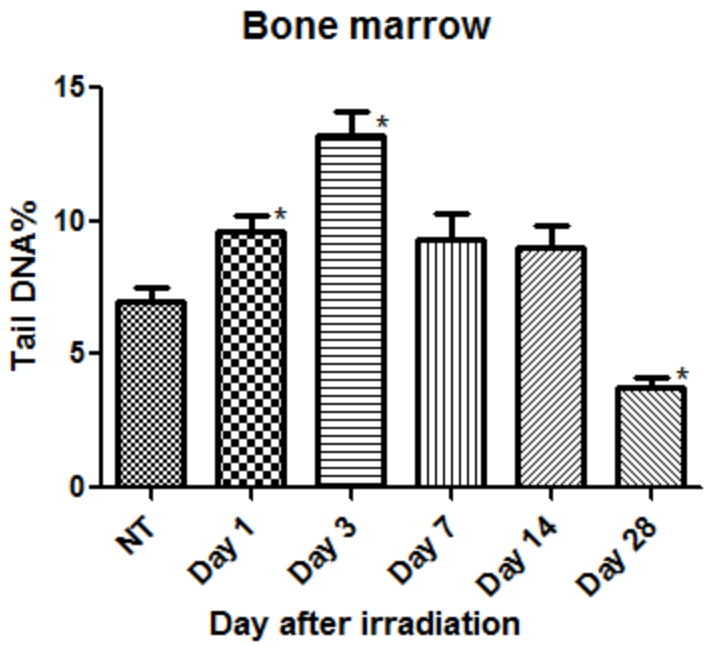
Cells of the mentioned tissues were isolated and the level of DNA damage by the comet DNA assay 28 days after irradiation.

It was shown that the maximum level of DNA damage in all studied samples is reached 3 days after irradiation, and repair is observed at 7-14 days after irradiation.

Effect of ultrashort pulsed electron beam irradiation in vivo







V.M. Tsakanov, et al.

AREAL low energy electron beam applications in life and materials sciences.

Nuclear Instruments and Methods in Physics. Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment

(2016), http://dx.doi.org/10.1016/j. nima.2016.02.028

Nelly Babayan, Galina Hovhannisyan, Bagrat Grigoryan, Ruzanna Grigoryan, Natalia Sarkisyan, Gohar Tsakanova, Samvel Haroutiunian, Rouben Aroutiounian.

Dose-rate effect of ultrashort electron beam radiation on DNA damage and repair in vitro.

Journal of Radiation Research, 2017.

https://doi.org/10.1093/jrr/rrx035

Babayan N, Grigoryan B, Hovhannisyan G, Tadevosyan G, Khondkaryan L, Grigoryan R, Sarkisyan N, Aroutiounian R.

Gender differences in DNA damage/repair after laser-generated ultrafast electron beam irradiation

Int J Radiol Radiat Ther. 2018;5(2):85–86.

Radiation-induced copy number variants

Tigran Harutyunyan, Galina Hovhannisyan, Anzhela Sargsyan, Bagrat Grigoryan, Ahmed H. Al-Rikabi, Anja Weise, Thomas Liehr, Rouben Aroutiounian

Analysis of copy number variations induced by ultrashort electron beam radiation in human leukocytes in vitro.

Molecular Cytogenetics. 2019. 12:18 https://doi.org/10.1186/s13039-019-0433-5

We are planning to get in near future the following results:

-The dose and dose-rate effects of ultrafast pulsed electron beam irradiation on DNA double-strand breaks formation in human tumor and normal cells will be studied.

-The detailed analysis of kinetics, the main ways of repair (non-homologous end joining and homologous recombination) and efficiency (residual damage) of double-strand DNA breaks repair in tumor and normal human cells irradiated with ultrafast pulsed electron beam will be realized.

-The tumor specific epigenetic alterations, based on methylation status and/or kinetics of core protein genes during the activation of specific DNA repair pathways will be investigated.

-Will be estimated the optimal mode of pulsed ultrafast electron beams, which causes the pronounced radiobiological effect.

Acknowledgements

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Best Wishes!