

# Visualization of complex DNA damage along accelerated ions tracks

*Elena Kulikova*<sup>1,2,\*</sup>, *Alla Boreyko*<sup>1,2</sup>, *Tatiana Bulanova*<sup>1,2</sup>, *Lucie Jeřková*<sup>1,3</sup>, *Mariia Zadneprianetc*<sup>1,2</sup> and *Elena Smimova*<sup>1</sup>

<sup>1</sup>Joint Institute for Nuclear Research, Dubna, Russia

<sup>2</sup>Dubna State University, Dubna, Russia

<sup>3</sup>University of Chemistry and Technology Prague, Prague, Czech Republic

**Abstract.** The most deleterious DNA lesions induced by ionizing radiation are clustered DNA double-strand breaks (DSB). Clustered or complex DNA damage is a combination of a few simple lesions (single-strand breaks, base damage etc.) within one or two DNA helix turns. It is known that yield of complex DNA lesions increases with increasing linear energy transfer (LET) of radiation. For investigation of the induction and repair of complex DNA lesions, human fibroblasts were irradiated with high-LET <sup>15</sup>N ions (LET = 183.3 keV/μm, E = 13MeV/n) and low-LET <sup>60</sup>Co γ-rays (LET ≈ 0.3 keV/μm) radiation. DNA DSBs (γH2AX and 53BP1) and base damage (OGG1) markers were visualized by immunofluorescence staining and high-resolution microscopy. The obtained results showed slower repair kinetics of induced DSBs in cells irradiated with accelerated ions compared to <sup>60</sup>Co γ-rays, indicating induction of more complex DNA damage. Confirming previous assumptions, detailed 3D analysis of γH2AX/53BP1 foci in <sup>15</sup>N ions tracks revealed more complicated structure of the foci in contrast to γ-rays. It was shown that proteins 53BP1 and OGG1 involved in repair of DNA DSBs and modified bases, respectively, were colocalized in tracks of <sup>15</sup>N ions and thus represented clustered DNA DSBs.

## 1 Introduction

The most significant biological effects of ionizing radiation (IR) are caused by the induction of a wide range of DNA damage in cells of living organisms. DNA lesions induced by the action of IR can be divided into individual and clustered DNA damage. Individual DNA damage includes a various types of base damage, single-strand breaks (SSBs), double-strand-breaks (DSBs) etc. Such DNA lesions are usually quickly and successfully recovered, while clustered DNA damage is difficult to repair or is even irreparable. Clustered DNA damage consists of two or more individual lesions within one or two helical turns and is formed especially after exposure to high-LET radiation [1]. Clustered DNA lesions can be either non-DSBs (any combination of two or more oxidized

---

\*Corresponding author: [kruglyakovaea@bk.ru](mailto:kruglyakovaea@bk.ru)

bases, strand breaks, or other DNA lesions that do not form a DSB) or DSBs clusters (DSB(s) in combination with any other DNA lesion). The pathways of individual DNA lesions repair are quite well known, however the exact repair mechanisms of clustered DNA damage give rise to a lot of questions. The most important of them are: 1) how does the presence of one lesion affect the efficiency of recognition and processing of the opposite/ neighboring lesion(s), 2) is there a hierarchy in repair of closely spaced DNA lesions, 3) which repair proteins participate in the processing of these lesions and what mechanisms are used by the cell to eliminate the presence of such lesions? [2]

The goal of this work was to investigate complexity and repair efficiency of clustered DNA double-strand breaks in normal human skin fibroblasts irradiated with accelerated  $^{15}\text{N}$  ions. Proteins involved into DSBs ( $\gamma\text{H2AX}$  and 53BP1) and DNA base damage (OGG1) repair were visualized using immunofluorescent staining procedure and high-resolution fluorescent microscopy. More detailed information about application of this method for studying of the processes of the DNA damage formation and repair can be found in this work [3].

It should be noted that the efficiency of the DNA DSBs repair induced by radiation of different quality based on the  $\gamma\text{H2AX}$  foci formation and elimination kinetics was also shown in [4]. However in this study only a qualitative analysis of the complexity of the induced lesions was performed. In current study a conclusion about higher complexity of DNA lesions induced by high-LET radiation in comparison with low-LET radiation was made based on a quantitative analysis of the  $\gamma\text{H2AX}/53\text{BP1}$  foci structure.

## 2 Material and methods

**Cell culture.** Primary normal human fibroblasts NHDF 22873 were maintained in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% fetal calf serum (FCS) and 1% gentamicin-glutamine solution (all reagents from Sigma-Aldrich, USA). Fibroblasts were grown at 37°C and 5%  $\text{CO}_2$ .

**Low-LET and high-LET irradiation.** Human fibroblasts were irradiated with low-LET  $^{60}\text{Co}$   $\gamma$ -rays and high-LET accelerated  $^{15}\text{N}$  ions in two geometries: 90° (perpendicular) or 10° (horizontal) angle between the ion beam and the plane of the cell monolayer (Table 1). Cells were irradiated with accelerated  $^{15}\text{N}$  ions using the isochronous cyclotron U-400M (Flerov Laboratory of Nuclear Reaction) and with  $\gamma$ -rays using the Rokus-M facility (Dzhelepov Laboratory of Nuclear Problems) at the Joint Institute for Nuclear Research (JINR, Dubna).

**Table 1.** Properties of exposure.

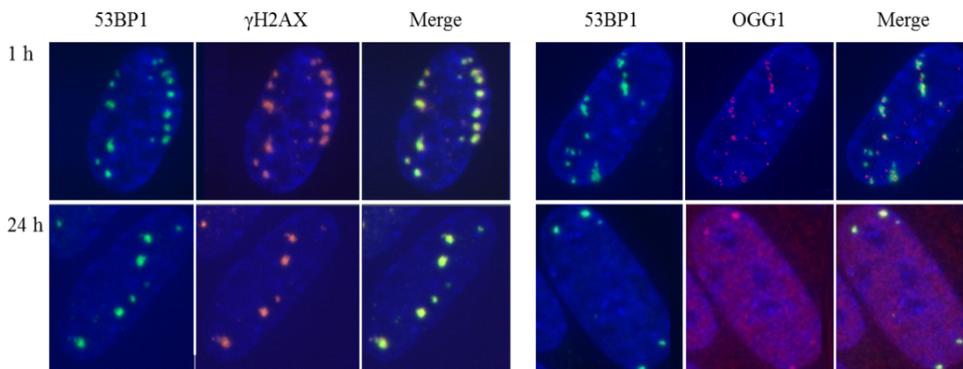
Radiation	Geometry	Energy, MeV/n	LET, keV/ $\mu\text{m}$	Dose, Gy	Facility
$\gamma$ -rays	90°	-	$\approx 0.3$	1	Rokus-M
$^{15}\text{N}$	90°	13	183.3	2.15	U-400M
$^{15}\text{N}$	10°	13	181.4	1.25	U-400M
$^{15}\text{N}$	10°	35	81.5	0.57	U-400M

**Immunostaining procedure.** At different time points after irradiation ranging from 5 min to 4 days, fibroblasts were fixed in 4% paraformaldehyde and underwent to standard procedure of immunostaining. For visualization of DNA DSBs ( $\gamma$ H2AX and 53BP1) and base damage (OGG1) markers, the specific primary and secondary antibodies were used (primary antibodies: anti-53BP1 rabbit polyclonal, anti-  $\gamma$ H2AX (phospho S140) mouse monoclonal (Abcam, USA); anti-53BP1 mouse monoclonal (Millipore, USA) and anti-OGG1 rabbit polyclonal (Novus Biologicals, USA) antibodies; secondary antibodies: Texas Red-conjugated goat anti-rabbit, FITC-conjugated goat anti-mouse and Alexa Fluor 488 conjugated goat anti-mouse (Abcam, USA)).

**Image acquisition and experimental data analysis.** Images of stained human fibroblasts nuclei were obtained using fluorescent microscope AxioImager.M2, equipped with optical light sectioning system ApoTome.2 (Zeiss, Germany). Quantitative analysis of colocalized 53BP1/ $\gamma$ H2AX foci was performed manually using the Acquarium software [5], which enabled the three-dimensional reconstruction of images and inspection of individual foci in 3D space (in x-y, x-z and y-z plane).

### 3 Results and discussion

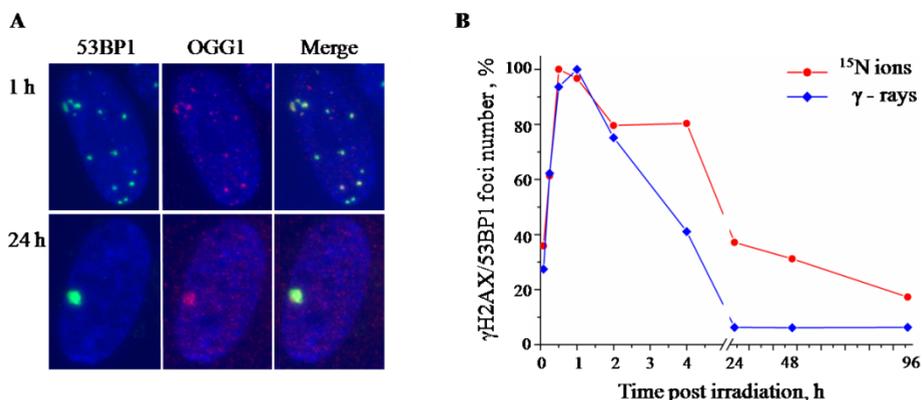
We successfully visualized DNA lesions in tracks of accelerated  $^{15}\text{N}$  ions (LET = 81.5 keV/ $\mu\text{m}$ , E = 35 MeV/n) in human fibroblasts irradiated at sharp-angle ( $10^\circ$ ) geometry. DNA double-strand breaks were visualized using colocalization of  $\gamma$ H2AX and 53BP1 foci (both markers of DSBs; Fig. 1. left panel) and clustered DSBs using colocalization of 53BP1 and OGG1 foci (markers of DNA DSBs and DNA base damage, respectively; Fig. 1. right panel). Analysis of the obtained images revealed that majority of 53BP1 foci were colocalized with OGG1 foci at all time points after irradiation and thus demonstrate that most of DNA DSBs induced by  $^{15}\text{N}$  ions were clustered. It was shown that 53BP1 and OGG1 foci were colocalized also after  $\gamma$ -irradiation and therefore we assume that these represent the clustered DNA DSBs (Fig. 2A).



**Fig. 1.** Visualization of DNA lesions in tracks of accelerated  $^{15}\text{N}$  ions (LET = 81.5 keV/ $\mu\text{m}$ , E = 35 MeV/n). **Left panel:** Visualization of DNA DSBs using markers 53BP1 (green) and  $\gamma$ H2AX (red). **Right panel:** Visualization of DNA DSBs and DNA base damage using markers 53BP1 (green) and OGG1 (red), respectively.

Assuming that the majority of DNA DSBs induced by both high- and low-LET radiation were clustered, we carried out quantitative analysis of  $\gamma$ H2AX/53BP1 foci in human fibroblasts irradiated in perpendicular geometry. Quantitative analysis revealed differences in the  $\gamma$ H2AX/53BP1 foci formation and elimination after irradiation with accelerated ions and  $\gamma$ -rays (Fig. 2B). Irradiation with the accelerated N ions (LET =183.3 keV/ $\mu$ m, E = 13 MeV/n) led to faster  $\gamma$ H2AX/53BP1 foci formation – the maximum number of foci was reached 30 min post irradiation (PI) while after  $\gamma$ -irradiation the maximum was reached within 1 h PI. Four hours post  $\gamma$ -irradiation, about 40% of the maximum numbers of  $\gamma$ H2AX/53BP1 foci still persisted in the cells while this fraction was 80% for accelerated  $^{15}\text{N}$  ions. After 24 h post  $\gamma$ -irradiation the number of  $\gamma$ H2AX/53BP1 foci reduced to 6% and remained at this level up to 96 h PI. Whereas in cells irradiated with  $^{15}\text{N}$  ions after 24 h PI the number of foci decreased to 37% and within 96 h reduced to 17%, which is almost 3 times greater than post  $\gamma$ -irradiation.

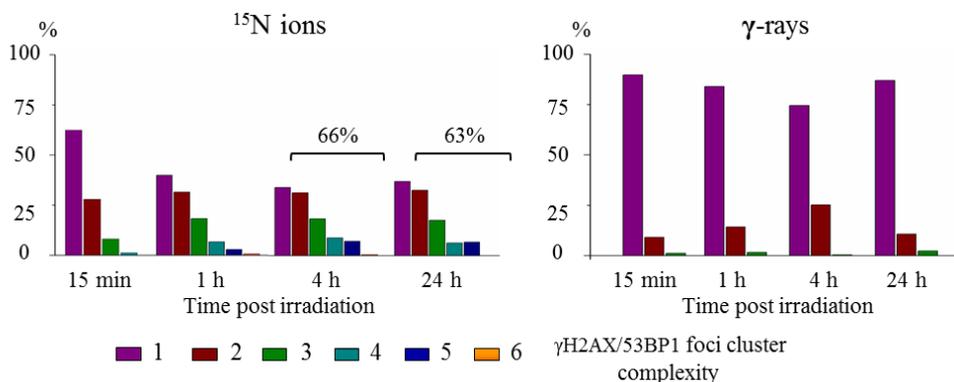
The obtained data clearly demonstrate slower repair of nitrogen induced DSBs suggesting much higher complexity of DNA DSBs compared to  $\gamma$ -rays. The slower repair kinetics may also indicate the involvement of specific repair mechanisms in the recovery of clustered DNA DSBs induced by high-LET radiation. There are two main DNA DSBs repair pathways in mammalian cells: non-homologous end joining (NHEJ) and homologous recombination (HR). Evidence indicates that NHEJ is the predominant repair pathway for DSBs induced by low-LET radiation [6], but there is a suggestion that this pathway of DNA repair may be only partially involved in repair of the clustered DSBs induced by high-LET radiation [7]. The colocalization of both markers of DNA DSBs and base damage repair (Fig. 1, 2A) shows the involvement in processing of clustered DSBs not only mechanisms of DSBs repair but also a base excision repair (BER) needed for base damage recovery. Thus, in the next step, involvement of the HR, NHEJ and BER in repair of clustered DNA DSBs induced by accelerated ions will be investigated.



**Fig. 2. A:** Visualization of clustered DNA DSBs after  $\gamma$ -irradiation: DNA DSBs – 53BP1 (green) and DNA base damage – OGG1 (red); **B:** Formation and elimination of  $\gamma$ H2AX/53BP1 foci after  $^{60}\text{Co}$   $\gamma$ -rays and accelerated  $^{15}\text{N}$  ions (LET =183.3 keV/ $\mu$ m, E = 13 MeV/n) irradiation in the perpendicular geometry.

Additionally the structure of radiation-induced  $\gamma$ H2AX/53BP1 foci in human fibroblasts irradiated by  $\gamma$ -rays and  $^{15}\text{N}$  ions (LET = 181.4 keV/ $\mu$ m, E = 13 MeV/n) at the angle of  $10^\circ$  was analyzed. The number of individual  $\gamma$ H2AX/53BP1 foci presented in clusters was counted, it was revealed up to 6 individual foci in one high-order  $\gamma$ H2AX/53BP1 foci

cluster in case of  $^{15}\text{N}$  ions irradiation (Fig. 3). The diagrams on Fig. 3 demonstrate the time dependent increasing of the  $\gamma\text{H2AX/53BP1}$  foci structure complexity after irradiation by accelerated ions, while the structure of  $\gamma$ -irradiation foci practically didn't change in time. It can be due to the induction of larger number of individual DSBs in one cluster induced by accelerated ions that is primarily caused by physical characteristics of the radiation. Preservation of the complex structure of  $\gamma\text{H2AX/53BP1}$  foci after 24 hours after irradiation suggest a difficulty in repairing of clustered DNA DSBs induced by accelerated  $^{15}\text{N}$  ions. Difficulty in repairing of clustered DNA DSBs may caused by the competition of several repair mechanisms since in addition to DNA DSB, as it was shown on Fig.1, the DNA base damage include in the clusters too.



**Fig. 3.** Comparison of  $\gamma\text{H2AX/53BP1}$  foci clusters complexity for  $^{15}\text{N}$  ions (LET = 181.4 keV/ $\mu\text{m}$ , E = 13 MeV/n) and  $\gamma$ -rays irradiation. The diagrams indicate the percentages of  $\gamma\text{H2AX/53BP1}$  foci presented in high-order clusters after 15 min, 1 h, 4 h and 24 h PI.

Thus, the issue of the cluster DNA damage repair is not simple and requires more thorough research and this will be continued in the following study.

## References

1. B.M. Sutherland, P.V. Bennet, H. Schenk, O. Sidorkina, J. Laval, J. Trunk, D. Monteleone, J. Sutherland. *Phys. Med.* **17** (2001) 202.
2. A.G. Georgakilas. *Mol. BioSyst.* **4** (2008) 30. doi: 10.1039/b713178j
3. D.V. Firsanov, L.V. Solovjeva, M.P. Svetlova, *Clin. Epigenet.* **2** (2011) 283.
4. F. Antonelli, A. Campa, G. Esposito, P. Giardullo, M. Belli, V. Dini, S. Meschini, G. Simone, E. Sorrentino, S. Gerardi, G. A. P. Cirrone, M. A. Tabocchini, *Rad. Res.* **183** (2015). DOI: 10.1667/RR13855.1
5. P. Matula, M. Maška, O. Daněk, P. Matula and M. Kozubek, *IEEE* (2009), 1138
6. A. Gerelchuluun, E. Manabe, T. Ishikawa, L. Sun, K. Itoh, T. Sakae, K. Suzuki, R. Hirayama, A. Asaithamby, D.J. Chen, K. Tsuboi. *Radiat. Res.* **183** (2015) 345.
7. S. Moore, F.K.T. Stanley, A.A. Goodarzi. *DNA Repair* **17** (2014) 64