USING NANOSCOPIC IMAGING OF DNA DSB REPAIR PROTEIN CLUSTERS AS A POTENT MARKER FOR BIOLOGICAL MICRODOSIMETRY ON HIGH-LET PARTICLE TRACKS IN HUMAN CELLS

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Ionizing radiation induces DNA double-strand breaks (DSB) with varying local density and complexity depending on its LET (Linear Energy Transfer). Based on the clinical relevance of the enhanced relative biological effectiveness of high-LET particle irradiation, it is of great importance to study the influence of radiation quality on the number of induced DSB. Also current evidence indicates that proteins, responsible for detection and repair of DSB, cluster in different structural and/or functional domains around the damage[1]. The aim of this study is to count every single DSB that is induced by high-LET irradiation.

We counted the number of ionizing radiation-induced DNA-PKcs foci (IRIF) in human HeLa cells 2-5 min after damage induction. Irradiation was performed at the ion microprobe SNAKE using high-LET 20 MeV lithium ions (LET=116 keV/µm), 27 MeV carbon ions (LET=500 keV/µm) and low-LET 21 MeV protons (LET=2.6 keV/µm). Imaging was performed using super-resolution STED nanoscopy with a resolution of 105 nm. The IRIF show an average size of 170±20 nm that allows separation of DSB by such small distances, i.e. in high-LET ion tracks.

DNA-PKcs IRIF label all DSB as proven by counterstaining with 53BP1 after low-LET proton irradiation where separation of individual DSB is in most cases larger than the 53BP1 gross size of about 0.6 µm. This enables counting of densely packed DSB in high-LET particle tracks due to the small IRIF size. Lithium ions produce 1.2±0.1 IRIF/µm track length, for carbon ions 2.2±0.2 IRIF/µm are counted. These results have been compared to Monte Carlo based simulations of ion-induced DSB with PARTRAC[2] yielding 2.7±0.4 DSB/µm for lithium and 10.2±2.2 DSB/µm for carbon ions; both are substantially higher than the IRIF measurements. However, when multiple DSB up to 150 nm or 200 nm distance were pooled in clusters that represent the size of the IRIF, the simulations matched the IRIF measurements: 1.25 and 1.4 clusters/µm for lithium ions, 1.8 and 2.2 clusters/µm for carbon ions, both for 200 nm and 150 nm cluster size, respectively.

In this study, it was possible to enhance present methods of DSB counting for high-LET particle tracks by a factor of 2-3. We conclude, that by counting the IRIF numbers it is possible to perform biological microdosimetry for high-LET particles. More details of the DSB distributions will be obtained after further reduction of the IRIF size.

[1] Reindl et al.;Sci.Rep. (2017)7:40616.

[2] Friedland et al.; Sci.Rep. (2017)7:45161.