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IRIF Cluster Formation and Structure in Human Fibroblasts after Irradiation with Boron Ions and γ-Rays

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Low-linear energy transfer (LET) radiation induces mainly isolated DNA lesions which are generally repaired efficiently. With increasing the LET value, damage complexity increases and clustered DNA double-strand breaks (DSBs) are formed. These complex DNA lesions are more difficult to repair than isolated ones or in some cases are irreparable. In this study, we have compared the formation of ionizing radiation-induced foci (IRIF) clusters and their structure in human fibroblasts irradiated with 1 Gy of 60Co γ-rays (LET ≈ 0.3 keV/µm) and accelerated 11B ions (LET = 137 keV/µm, E = 8.4 MeV/u), using immunostaining for DNA DSB response proteins γH2AX and 53BP1. Sample irradiation with 11B ions was done in two geometries: perpendicular to the beam direction to study the kinetics of γH2AX/53BP1 foci formation, and at a small angle (10°) between the beam direction and cell monolayer to analyze the foci distribution along the particle trajectories. High-resolution confocal microscopy and 3D reconstruction of cell nuclei allowed us to analyze in detail the structure of clustered γH2AX/53BP1 foci. The obtained data show differences in the kinetics of γH2AX/53BP1 foci formation and elimination in cells irradiated with γ-rays and 11B ions. Under the action of accelerated boron ions, the number of γH2AX/53BP1 foci decreases slower than for γ-irradiation. Within 4 h after γ-irradiation, most of the foci are eliminated, whereas the elimination of ~80 % of the foci is achieved 24 h after 11B ion irradiation. A detailed analysis of foci cluster structure demonstrates that the complexity of the 11B ion-induced clusters is higher and more variable. In the cells irradiated with 11B ions, more than 80% of the foci form clusters comprising several individual foci in mutual contact, whereas only ~ 30% of the foci were identified as clusters after γ-irradiation. It is observed that the fraction of more complex clusters increases with post-irradiation time. The increasing multiplicity of γH2AX/53BP1 clusters probably reflects the irreparability of the highly complex clusters. Also, a dose dependence of γH2AX/53BP1 foci formation 1 h after 11B ions irradiation was obtained.

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