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Microtubules as IR target

Radiotherapy is a rapidly evolving domain encompassing various aspects, including the utilization of diverse beam types (protons, heavy ions, X-rays, electrons) and delivery methods, such as the emerging FLASH modality. To enhance clinical decision-making, a comprehensive understanding and modeling of physical, biophysical, and biological processes are essential. Initial and pivotal investigations often involve monolayer cell cultures. We study the microtubule (MT) network as a target of Ionizing Radiation (IR), in addition to DNA, traditionally recognized as the primary target of IR-induced damage. MTs present novel avenues for modeling. Our focus lies on studying MT occupancy and curvature, which correlates with the acetylation mechanism of lysine amino acid at position 40 of α-tubulin.

We acquired 3D STORM images employing a SAFe360 module (Abbelight) integrated with an inverted bright-field Olympus IX71 microscope, achieving a resolution of approximately 20nm. Confocal microscopy images were obtained using the Laica Stellaris 5 system. Subsequently, STORM and confocal images were analyzed using ImageJ software for filament reconstruction and curvature calculation. Statistical analyses were conducted using MATLAB.

We analyzed STORM and confocal images of non-cancerous and cancerous human mammary cells irradiated with different beam types protons vs. X-rays in a dose range from 4 to 15 Gy. The analysis aims to quantify MT area occupancy, perform filament reconstruction, and assess MT curvature. Statistical tests were conducted for each population. From the results it is clear that super-resolution microscopy techniques could bridge molecular integrity with geometric and spatial properties.

This is particularly significant as MTs, comparable in size to DNA, are viable candidates for energy distribution simulations using similar computational approaches. These findings offer insights into the fundamental mechanisms governing cell proliferation and survival.

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