STUDY OF EARLY DNA DAMAGE RESPONSE DYNAMICS AFTER CHARGED PARTICLE IRRADIATION BY BEAMLINE MICROSCOPY

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Approaches to visualise the dynamics of DNA lesion processing substantially contribute to the understanding of the hierarchies of the DNA damage response pathways. Charged particle irradiation has recently emerged as a tool to generate discrete sites of subnuclear damage by means of its extremely localised dose deposition, thus facilitating the spatiotemporal analysis of repair events. In this regard low energy particles share similarity to the now widely used UV-laser microirradiation, with the advantage of a defined dose deposition and ionizing radiation properties similar to x- or γ-rays. In addition, the special physical and biological properties of accelerated charged particles are of great interest for the application in advanced radiation therapy of solid tumours and, as one of the major contributions of cosmic rays, for risk estimation of human space exploration (mars mission).

A major challenge for the microscopy of early radiation effects with charged particles arises from the fact that the whole setup has to be controlled remotely during irradiation due to radiation safety regulations. The actual setup of the GSI beamline microscope consists of a modified Olympus IX71 frame, a piezo focusing device (pifoc, Physik-Instrumente), a PolyV monochromator (Till-photonic) and an Andor iXon EMCCD camera as key parts. The microscope frame has to be turned by 90° to be adapted to the horizontal beamline. The cells were cultivated on thin polymer foils and irradiated in medium filled chambers. Microscopic imaging was done with a 60x NA1.2 water immersion lens through 250 µm of cell medium.

As a major application, the beamline microscopy allows determining the exact kinetics of fluorescently tagged repair proteins after irradiation with different charged particles inducing different lesion densities. The classification into fast recruited proteins like DNA-PK or XRCC1 or slower recruited ones like 53BP1 or MDC1 helps to establish the hierarchical organisation of damage recognition and subsequent repair events [1]. Additionally, motional analysis of DNA lesions induced by traversing particles provided information about the mobility of DSBs. Increased mobility might have direct consequences on the formation of chromosomal translocations and thus on the probability of cancer formation.

Recently an 478 nm solid state laser for photobleaching experiments (FRAP) was coupled to the beamline microscope allowing to access the exchange and binding of the recruited repair proteins directly after irradiation with charged particles.

In conclusion, the present version of the beamline microscope provides a tool to contribute to several open fields in radiation biology and DNA repair which will be discussed. Introducing small modifications, it was recently demonstrated that the device can also successfully be used at the high energy branch of the accelerator (1GeV/u) mimicking cosmic high energetic charged particles.


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