ULTRA HIGH RESOLUTION FLUORESCENCE MICROSCOPY TO ANALYSE THE FINE STRUCTURE OF ION INDUCED DNA REPAIR FOCI

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Ionizing radiation creates double-strand breaks (DSB) of high local density and different complexity with respect to its LET (Linear Energy Transfer). There are several repair factors that accumulate into so called “repair-foci” of about 1 µm or smaller in diameter. The kinematics of foci formation, the foci movement dynamics and its fine structure is of our actual interest in order to understand DNA repair process and the different ingredients of DNA mis-repair. The actual study is dedicated to analyse the fine structure of the repair foci by optical, super-resolution fluorescence microscopy utilising STED (Stimulated Emission Depletion) that provides a resolution better than 100 nm. A commercial STED microscope (LEICA TCS SP8) was acquired and installed at the Universität der Bundeswehr München. The intention is to analyse the fine structure of different repair factors stained in one sample and to correlate the structures. For first experiments we irradiated HeLa cells under a flat angle to the image plane by 21 MeV protons and 55 MeV carbon ions in order to compare foci structures from high and low LET irradiation. The 55 MeV carbon ions lose energy in the polypropylene foil which is used as a growing substrate for the cells and thus the energy decreases to 36 MeV when penetrating the cells. Thus, we obtain LET values of LET=2.56 keV/µm for the protons and LET=416 keV/µm for the carbon ions.

Figure 1 shows images of a HeLa cell nucleus that was irradiated with 36 MeV carbon ions. Different damage markers are labelled, like 53BP1, Rad51 and γH2AX. In a) and b) Rad51, which shows several small dot like foci with a gross size of 200 nm is labelled in green. The overlay with the almost continuous track of 53BP1 (Fig. 1a) shows that Rad51 covers the intensity minima inside the 53BP1 tracks, which occur due to a 53BP1 fine-structure. The same behaviour can be reveald by a superposition of Rad51 and γH2AX (Fig. 1b).
The comparison of 53BP1 and γH2AX (Fig. 1c) shows an inner structure of both 53BP1 and γH2AX tracks. These structures are not the same for the both repair proteins and there is only partial overlap, if any.
In Figure 2 a HeLa cell is presented, which was irradiated with 21 MeV protons at 21 Gy average dose. The comparison with Figure 1 shows that now no continuous ion tracks for 53BP1 are visible but only distinct foci, which, however, still express an inner structure. Again, the Rad51 Foci are located within the 53BP1 foci at intensity minima.

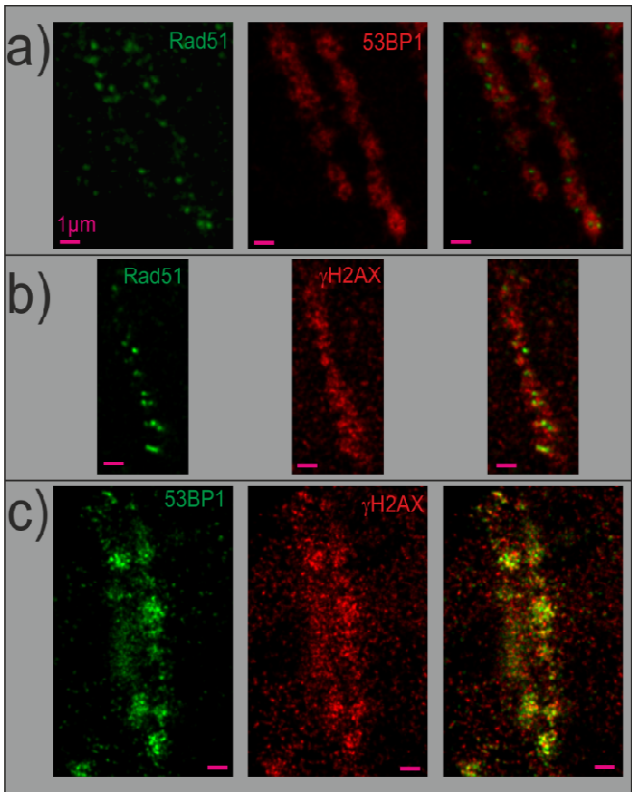

Figure 1 shows extracts of HeLa cells irradiated with 36 MeV carbon ions. a) presents two ion tracks labelled with Rad51(green) and 53BP1(red). b) shows one ion track where rad51 (green) and γH2AX (red) are labelled. In c) two tracks with 53BP1 (green) green and γH2AX (red) are displayed.

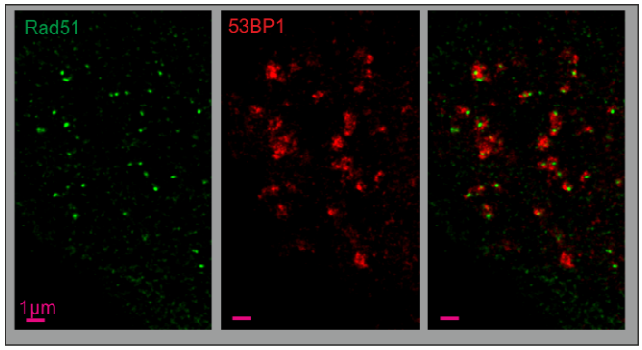

Figure 2 shows a HeLa cell irradiated with 21 MeV protons. Rad51 is labelled in green and 53BP1 is labelled in red