Dynamics of Irradiation Induced Foci of γ-H2AX in Living Cells

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The irradiation setup installed at the ion microprobe SNAKE is capable of irradiating living cells with submicron resolution using high energy ions [1,2]. Now for the first time quantitative experimental results can be presented [3]. In order to study the dynamics of DNA repair processes living HeLa cells were irradiated with a line pattern consisting of single 100 MeV oxygen ion hits separated by 1 μm. At the sites of DNA-double strand breaks (DSBs) induced by the ions various proteins accumulate which are more or less directly involved in DNA repair processes. These clusters of proteins form so-called foci which can be made visible under the fluorescence microscope by immunostaining techniques.

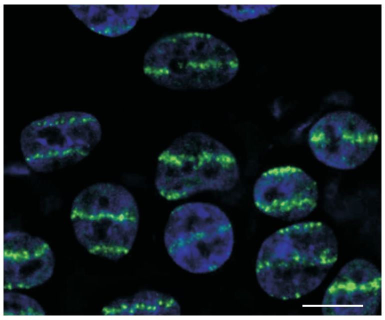

Fig. 1: Cells 0.5 h after irradiation. Blue: DAPI stained cell nuclei, green: γ-H2AX foci. (Scale bar 10 μm)

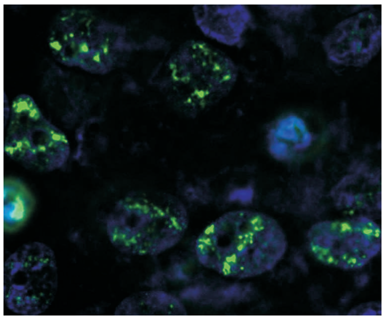

Fig. 2: Cells 15 h after irradiation.

To study the time-resolved dynamics of DNA repair processes, several cell samples were irradiated and incubated for various periods of time before fixation. As seen in figure 1, foci formed by the phosphorylated γ-H2AX reproduce the irradiation pattern nicely when the cells were fixed shortly after irradiation. If, however, the cells are kept for 15 h after irradiation, the foci appear clearly dispersed around the irradiated line pattern. This reflects the mobility of damaged chromatin regions within the nucleus.

To quantitate these movements, the brightness of the fluorescence signals was determined along lines perpendicular to the irradiation line. After fitting these data with a Gauss function, the deviation σ of the centres of mass of the foci around the irradiated line was evaluated. The increase of σ with time, which reflects the dispersion of the foci, is shown in figure 3.

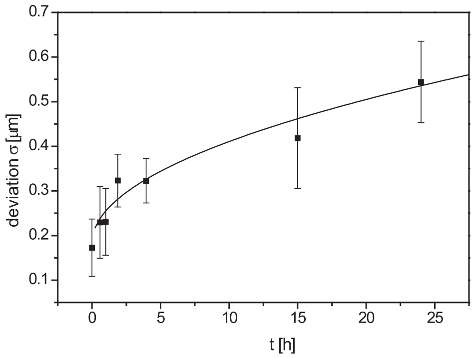

Fig. 3: Scattering of the foci around the irradiated line.

The data are compatible with a model assuming diffusion. Fitting the data with a function $\sigma \propto \sqrt{Dt}$ results in a diffusion constant of $D = (2.6 \cdot 10^{-3} \pm 1.4 \cdot 10^{-3})\, \mu m^2/h$.

In addition to foci dispersion, also foci size exhibits a time-dependent variation. To determine foci size, the medial line width, which correlates with the foci size, was determined via the FWHM of the Gaussian fit.

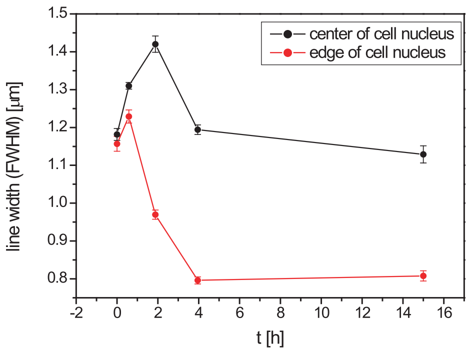

Fig. 4: Temporal trend of line width.

Figure 4 shows that the line width initially increases, which probably reflects ongoing posphorylation of γ-H2AX in the surrounding of the damaged site. Then, a decrease in foci size is observed, the reason of which has not yet been elucidated. At current, it is tested whether this decrease is due to chromatin compaction in the vicinity of damaged sites.

References

[1] Annual report 2003, p. 60.
[2] A. Hauptner *et al.*, Radiat Environ Biophys **42** (2004) 237-245.
[3] V. Hable, diploma thesis, TU München, 2004.