

Direct evidence for the spatial correlation between individual particle traversals and localized CDKN1A response induced by high-LET radiation

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In a recent study, we showed that heavy ion irradiation of human fibroblasts leads to an extremely localized nuclear response of the CDKN1A (p21) protein (*1*). The number of CDKN1A foci per cell nucleus after heavy-ion irradiation suggested a direct correlation with the number of particle traversals and therefore also a spatial correlation between foci and particle traversals. However, this conclusion was essentially based on statistical arguments, comparing the number of foci with the average number of particle hits which are expected at a given fluence and geometrical size of the nucleus.

But using a method based on retrospective detection of traversals in nuclear track detection material, we were now also able to directly assign the individual foci to the sites of individual particle traversals (2,3). The procedure of fluorescence imaging and track image matching includes the following steps:

1. Cells are grown on CR39 plastic material which is used as a nuclear track detector. Individual particle tracks can be made visible in this material by etching in NaOH.
2. Cells are irradiated and immunostained using a CDKN1A antibody as described previously.
3. Images of the nuclei showing CDKN1A foci are taken and the positions of the cells are stored using a computerized microscope stage control (image type I). At the same time, phase contrast images are taken at the same position (image type II).
4. After taking the images of CDKN1A foci, cells are removed from the CR39 plates, and the plates are etched for track detection.
5. The plate positions for which images of CDKN1A foci were taken are relocated and images are taken at the same position, showing now the pattern of particle traversals (image type III). They can be distinguished from the reference tracks by their smaller size.
6. By comparing the different images, the spatial correlation of tracks and foci is determined. Possible displacements due to repositioning inaccuracies can be detected by means of reference tracks, which are produced by pre-irradiation of the CR39 with charged particles.

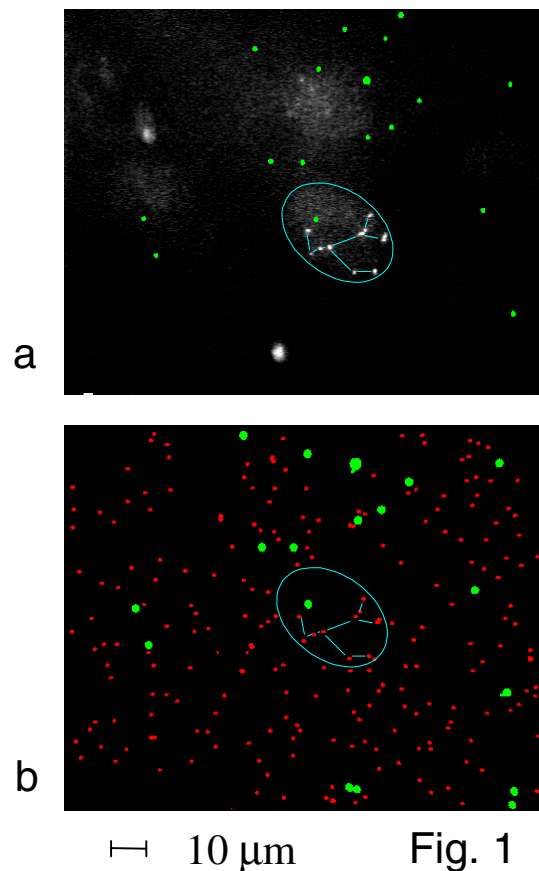
Images were then further processed to facilitate the analysis. First, images of type I were optimized by manually adjusting the contrast. Secondly, images of type II were transformed using a threshold procedure to specifically select only the reference track structures. The resulting images are then combined using a false color coding, so that the reference tracks appear in green, whereas the fluorescence intensity of the CDKN1A signal remains as a gray scale pattern (Fig. 1a). Images of type III were segmented also according to a manually adjusted threshold, and the corresponding objects are subsequently classified according to their size into two groups. Again, color coding was chosen as green for the reference

tracks, whereas the tracks corresponding to the cell irradiation are coded in red.

In Fig. 1, light blue lines indicate the important structures. An ellipse represents the approximate shape and size of the cell nucleus. Straight lines interconnect individual CDKN1A foci in order to enhance the particular pattern. The same pattern is shown in the lower panel (Fig. 1b) as overlay to the track image, and the patterns of CDKN1A foci and particle traversals coincide precisely.

A similar coincidence was found for all but one of about 35 cells analyzed in this experiment. Furthermore, the same type of correlation has been found for other proteins like e.g. hMre11 (4).

These results thus represent further confirmation that the response of CDKN1A and hMre11 on the microscopic level reflects the typical spatial energy deposition pattern of charged particles and thus the distribution of primary induced damage.



References:

- (1) B. Jakob et al., Rad. Res. 154, 398-405 (2000)
- (2) C. Soyland and S.P.Hassfjell, Rad. Env. Biophys. 39, 125-130 (2000)
- (3) M. Scholz et al., Rad. Res. 156, 558-563 (2001)
- (4) B. Jakob et al., Int. J. Radiat. Biol. 78, 75-88 (2002)