Colocalisation Analysis of Irradiation-induced Foci of DNA Repair Proteins after Ion-microirradiation

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With the ion-microbeam SNAKE (*S*uperconducting *N*anoprobe for *A*pplied Nuclear (=*K*ern-) Physics *E*xperiments) at the munich tandem accelerator facility, single ions can be applied in geometrical patterns to living cells. Thereby, DNA damage induced by ionizing radiation is directed with a targeting accuracy of less than 1 μm to a certain subset of cells or specific areas in single cells. With microirradiation, DNA double strand breaks are induced in a well defined manner and the formation of irradiation induced foci (IRIF) can be visualized after fixation of cells by using immunofluorescence techniques.

We analysed different candidate proteins involved in DNA repair for accumulation at sites of DNA damage. The histone variant H2AX has been shown to be phosphorylated in response to DNA double strand breaks (DSBs) (designated as γ-H2AX) and is a widely used marker for DSBs. To determine colocalisation of candidate proteins, e.g 53BP1 (p53 binding protein 1), with γ-H2AX (fig. 1), we performed intensity correlation analysis (ICA), which has been previously described by Li et al. [1]. This analysis uses the PDM (product of the differences from the mean) values for pixel intensity. The PDM is calculated from $(A_i - a)(B_i - b)$, where A_i and B_i represent the pixel staining pair intensities for two channels and a and b are the respective means of pixel intensities for the whole image. The ICA can be performed by using an automated graphic plug-in (intensity correlation analysis) for the public image processing software ImageJ. This plug-in allows PDM mapping showing two ICA plots, where A_i or B_i are plotted against their respective PDM values. If two fluorochromes colocalize (dependent staining), their intensities in a pixel will vary in synchrony and the PDM values will skew to the right ($PDM > 0$). Symmetrical distribution about the zero axis represents random staining ($PDM \approx 0$), while those skewing to the left indicate for contra-localization (independent staining; $PDM < 0$). A PDM color code shows the PDM values for the nucleus, where the scale on the left represents the color code for positive and negative PDM values, respectively. Colocalization can be estimated also in a quantitative manner by using the intensity correlation quotient (ICQ). ICQ is calculated from the proportion of pixels with $PDM > 0$ reduced by 0.5 to vary from 0.5 to –0.5 (complete colocalization and contra-localization, respectively). ICQ is threshold dependent, as PDM depends on mean intensities [2].

References

[1] Q. Li *et al.*, J Neurosci 2006.
[2] O. Ronneberger *et al.*, Chromosome Res 2008.

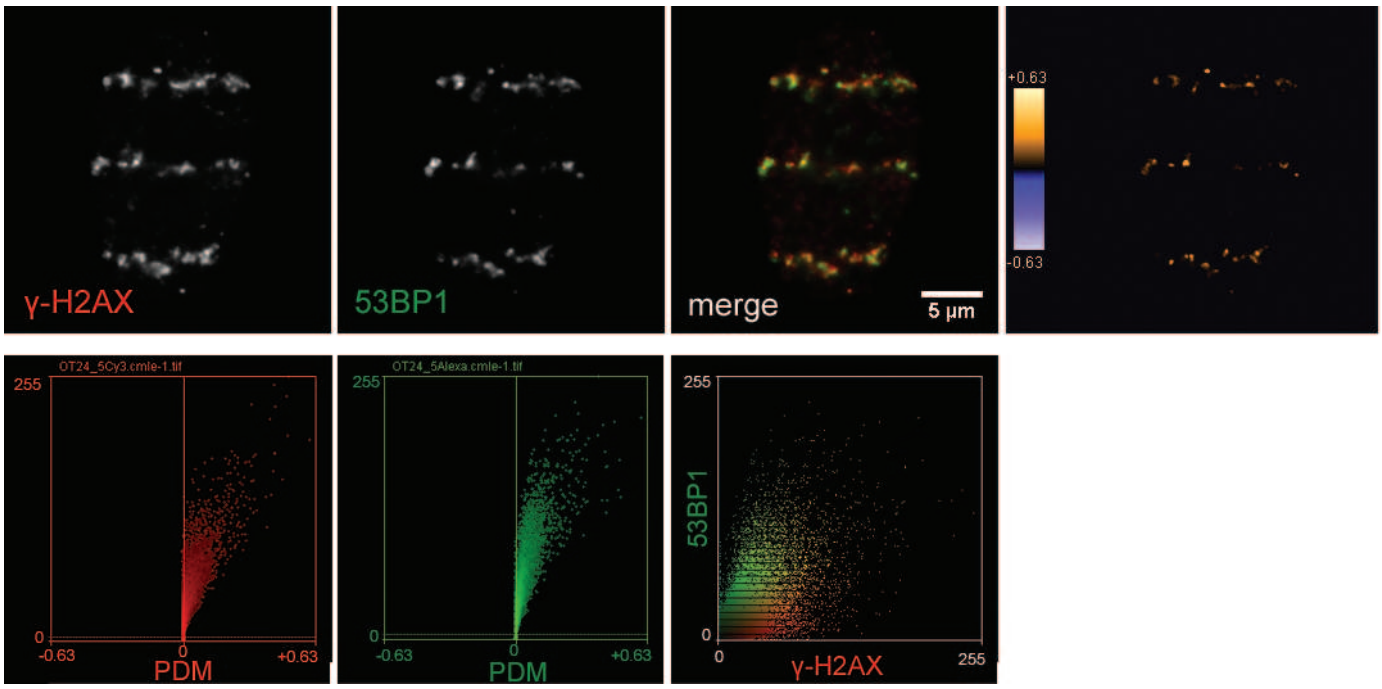

Fig. 1: An example of colocalization analysis: Upper row shows z-projections of the nucleus after immunofluorescence staining of γ-H2AX and 53BP1 and a merged image after ion-microirradiation. HeLa cells were irradiated at SNAKE with accelerated carbon ions in linear patterns. Image on the right shows PDM mapping of the nucleus. Color code for PDM values is shown in the scale. Regions where intensities are strongly correlated are highlighted in yellow. As there are no brighter blue stainings in the image, this demonstrates the high synchrony of the two fluorochromes. Lower row: ICA plots for the two channels. PDM values are in the positive range indicating colocalisation (ICQ = 0.277). On the right the color scatter plot shows combinations of intensities of both channels and their color.