

Detection of radiation induced DNA damage using quantum dot coupled antibodies*

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Introduction

Resolution in light microscopy is generally limited by diffraction, even if new approaches start circumventing this barrier by either narrowing the excitation point spread function or the localization of single chromophores. Traditionally, electron microscopy covered the realm of higher resolution, but at the cost of more sophisticated preparation of biological samples. Especially difficult is the observation of structures in the interior of the cell nuclei, which need either mechanical sectioning, thus sacrificing the 3D context as in transmission electron microscopy (TEM) [1], or rupturing for scanning electron microscopy (SEM), which is able to provide images of the 3D topology of the sample. However for the detection of epitopes by immunocytochemistry, standard preparation conditions present a hurdle for the delivery of bulky antibody (AB) conjugates. In this study, we established fixation and permeabilisation conditions to deliver quantum dot coupled antibodies to DNA double strand breaks induced by charged particle irradiation under a low angle [2]. These AB-bound quantum dots have a similar size (around 10 to 20 nm) as gold beads, the standard detection method of antigenic structures in SEM.

Materials and Methods

NIH-3T3 cells were cultured and irradiated as described [2]. Irradiation was done using uranium ions (4.7 MeV/u, LET \approx 15000 keV/ μ m). Cells were extracted and fixed using Streck tissue fixative as described [3]. Additional permeabilisation using methanol was added after fixation. For immunocytochemical staining γ -H2AX (Millipore) monoclonal-AB was used. Samples were stained sequentially with two different secondary ABs carrying either an organic chromophore (Alexa 488, green) or qdots 625 (red). Fluorescence microscopy was done at a Leica SPE confocal utilizing a 63x 1.3 NA oil lens.

Results

The principle aim of this study was to establish fixation and permeabilisation conditions allowing the delivery of large (10-20 nm) sized probes to internal structures of cells after irradiation, which is a prerequisite for their detection by immunogold-

labelling in SEM. Using common formaldehyde based fixation resulted in an exclusion of quantum dots from the interior of cell nuclei in combination with unspecific binding at the outer surface (not shown). Applying prefixation extraction steps in combination with diazolidinyl urea/2-bromo-2-nitropropane-1,3-diol based fixation and Tritonx-100 permeabilisation led to an uptake and binding of quantum dot-labelled antibodies to the targeted structure. The penetration and binding could be further improved using post-fixation permeabilisation by MeOH (Fig. 1 right). Specific labelling could be demonstrated by double-staining with conventional Alexa 488 labelled ABs (Fig.1 left). The bright stripes in the nuclei represent γ -H2AX at DNA DSBs induced by the traversing ions.

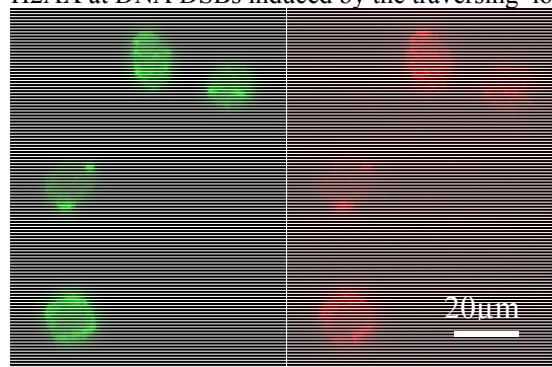


Figure 1: Fluorescence image of NIH-3T3 cell nuclei irradiated with low energy uranium ions. Stripes indicate DNA double strand breaks along the ion trajectories. Left (green) panel shows the organic chromophore Alexa 488 bound to γ -H2AX. Right panel (red) shows the AB-coupled qdots binding to the same primary AB thus indicating an adequate delivery of larger probes to the internal damage sites.

Conclusions

An improved fixation method was established allowing the specific labelling of radiation-induced changes inside the cell nucleus using relatively large probes otherwise excluded. In the next step, this method will be applied to secondary antibodies coupled to 10 nm gold beads at the SEM.

Reference

- [1] Y. Lorat et al., DNA Rep., (2015) epub.
[2] B. Jakob et al., Rad. Res., 159; (2003) 676.

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