High Resolution Imaging of Radiation Induced Changes in Chromatin Structure using DRAQ5 assisted DAB Polymerization and TEM

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DNA is generally considered to be the main target for cellular damage induced by ionizing radiation. In mammalian cells repair of DNA lesions is governed by the underlying chromatin organization and its structural changes which are induced to facilitate damage processing and signaling. If cells were exposed to radiation with high ionizing density (linear energy transfer, LET) like charged particle irradiation, clusters of complex double strand breaks (DSBs) are likely to occur, which might induce large amount of chromatin changes during damage processing. After irradiation with charged particles a local decondensation of heterochromatin has already be described to occur along the ion trajectory using fluorescence microscopy [1] or electron microscopy (EM) [2]. The latter allows addressing chromatin architecture at very high resolution. In [2] uranyl acetate (UA) was used to contrast the sample for EM, but UA binds not selectively to DNA but rather to lipids, proteins and RNA as well. Recently, a method was published to specifically contrast DNA by use of localized 3,3'-Diaminobenzidine (DAB) polymerization upon the production of singlet oxygen by the fluorescent DNA binding dye DRAQ5 [3].
Here, we established the polymerization of DAB in different cell lines expressing GFP-tagged DSB repair factors. After irradiation and fixation with glutaraldehyde the cells were first inspected for DNA DSBs in a fluorescence microscope (FM) and regions of interest (ROIs) were defined for high resolution EM analysis. Afterwards DAB polymerization and OsO4 staining were done. By the aid of a grid in the glass bottom of the petri dish which gets imprinted into the resin block it was possible to correlate the fluorescence and EM images and revisit the ROIs. First results on radiation induced chromatin changes using this method will be discussed.

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