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Detection of chromatin decondensation induced by charged particle irradiation using Fluorescence Lifetime Imaging Microscopy*

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Introduction

Advances in a variety of photonic imaging techniques and development of fluorescent proteins make Fluorescence Lifetime Imaging Microscopy (FLIM) a promising technique for quantitative biophysical measurements. The lifetime of excited electronic states are largely independent of the dye concentration and can provide insight into the local environment as well as dynamics of biological processes. Using widefield microscopy, we showed that ion irradiation induced a local decondensation of heterochromatin at the sites of ion hits in murine chromocenters, which is accompanied by a relocation of the induced double-strand breaks (DSB) to an adjacent euchromatin [1]. Here, to establish a chromatin compaction sensitive probe, we measured the lifetime of DNA binding dyes in ion irradiated and subsequently fixed mouse cells.

Materials and Methods

NIH-3T3 cells were cultured, irradiated and fixed as described in [2]. Irradiation was done using uranium ion (4.7 MeV/u, LET ≈ 15000 keV/µm). For immunofluorescence staining XRCC1 mouse monoclonal and Alexa Fluor 514 anti-mouse antibodies were used. A microscope (Olympus IX71; lens: 60x 1.2 NA water) coupled to a DCS-120 confocal scanner, picosecond diode lasers (405 nm, 445nm, 515 nm) and hybrid detectors (HPM-100-50) (all bh GmbH) were used to produce confocal FLIM images.

Results

The principle aim of the present study was to establish a FLIM setup by which radiation-induced chromatin decondensation in murine cells can be monitored. Up to now, attempts quantifying hetero-chromatic decondensation have been based solely on intensity information [1, 2]. The new FLIM approach considered intensity independent changes in the decay velocity of the excited states due to chromatin density dependent quenching in addition. To test our system, we irradiated NIH-3T3 cells with uranium ions and fixed them by 2% formaldehyde. The site of ion traversals within the chromocenter was determined by the aggregation of the damage marker XRCC1 (Figure 1, panel C). Different DNA dyes were screened. A promising probe is Hoechst 34580 as it shows a reduction of fluorescence intensity corresponding with an increase in lifetime at sites of heterochromatic ion traversal (Figure 1). The profile is calculated by radial angle integration on hit chromocenter indicated by blue crossed lines in Figure 1 panel A and B.

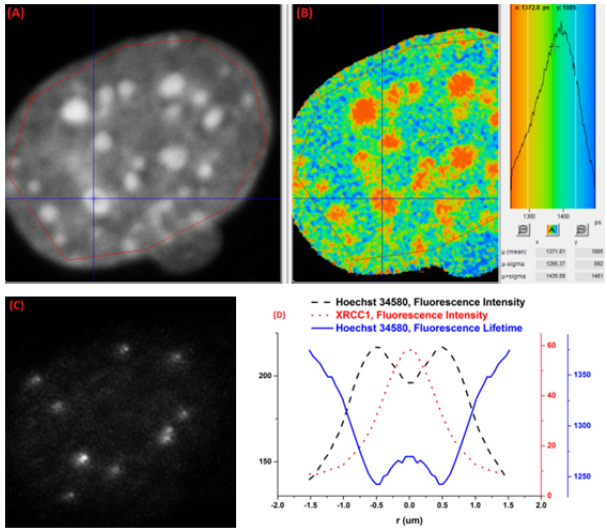

Figure 1: Confocal FLIM image of irradiated and fixed NIH-3T3 cells stained with 1 µM Hoechst 34580. Intensity and lifetime distribution of Hoechst 34580 images depict in panel (A) and (B). Panel (C) Alexa 514 bound to XRCC1 as indicator of ion hits. Panel (D) intensity and lifetime profiles representing depleted DNA staining (dash: intensity and solid: lifetime) at the damage site marked by XRCC1 (dot).

Conclusions

The results of this study indicate that the established FLIM setup is capable of monitoring radiation-induced chromatin decondensation in fixed murine cells. Since the DNA dye Hoechst 34580 reveals clear lifetime discrimination between condensed and non-condensed areas, it will be further characterized in order to use it for the measurement of real time kinetics of radiation-induced chromatin decondensation in living cells during irradiation at the beamline.

References

[1] B. Jakob et al., NAR, 39, (2011) 6489.
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