S PHASE SPECIFIC RESPONSES OF THE DNA REPLICATION AND REPAIR MACHINERY AFTER ION MICROBEAM IRRADIATION

S.Smith[1], A. Gleiche[1], S.E. Drexler[2], C. Greubel[3], J. Reindl[3], S. Girst[3], C. Siebenwirth[3], G.A. Drexler[2], G. Dollinger[3], A.A. Friedl[2], F. Grosse[1] and H. Pospiech[1]

[1] Biochemistry Group, Leibniz Institute for Age Research – Fritz Lipmann Institute, Jena, Germany, [2]LMU Department of Radiation Oncology – Ludwig Maximilian University, München, Germany, and [3]Angewandte Physik und Messtechnik LRT2, UniBw-München, 85577 Neubiberg, Germany

Abstract

Selected proteins involved in the Damage Response (DDR) pathway or in DNA replication were investigated to elucidate the interaction of these pathways in response to Irradiation (IR) damage. Using the SNAKE microprobe and a novel method of cell cycle analysis in Immuno-fluorescence (IF), known as hiMAC, the dynamics and localization of the essential human DDR and replication factor TopBP1 to the damage site was followed after irradiation with single 55 MeV carbon ions, as a function of the cell cycle phase.

INTRODUCTION

Highly proliferative and rapidly dividing cells are seen to be highly IR sensitive [1]. However, cells are more resistant to this damage in S phase compared to other cell cycle stages. This limits the success of IR therapy in the treatment of tumours, as many cancerous cells rapidly proliferate, thus giving them a higher resistance to this type of treatment [2]. Therefore, understanding the interaction between the DNA replication apparatus and the DNA damage response to IR damage, particularly for the repair of DNA double-stranded breaks, is important to understand biological irradiation damage in more detail.

MATERIALS AND METHODS

Human U2OS osteocarcinoma cells were irradiated at low angle to the image plane with 55 MeV carbon ions at SNAKE microprobe. Cells were allowed to recover from DNA damage for 20 minutes to 2 h post irradiation. Growth medium was supplemented with ethynyl-deoxyuridine (EdU) 15 minutes before fixation. Afterwards, samples were processed for IF using antibodies against γH2AX and TopBP1. DNA synthesis was visualised by click coupling of the incorporated EdU with AF647 azide [3]. Images from the irradiated zone were acquired at 400x magnification using the BD pathway 435 High Content Bioimager. Image analysis was based on the hiMAC method [4], using Cell Profiler 2.0, in order to identify the cell cycle stage and damage tracts.

RESULTS

DNA damage tracks of single 55 MeV carbon ions were visible as γH2AX (green) tracks between 20 min and 2 h post irradiation from images acquired by automated high content fluorescence microscopy and processing (Fig. 1).

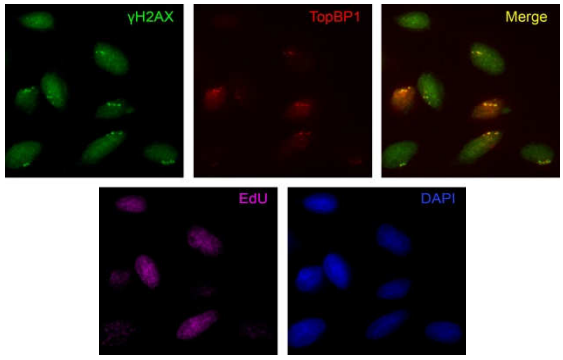

Figure 1. Sample images from the BD pathway, two hours after low angle irradiation with 55 MeV carbon ions.

DNA synthesis and DNA intensity (DAPI) was used for IF-based cell cycle staging (Fig. 2, left). Most cells within the irradiated zone displayed accumulation of TopBP1 to the DNA damage (γH2AX) tracks. However, TopBP1 level in the damage tracks declined faster over time in G1 nuclei (Fig. 2, right).

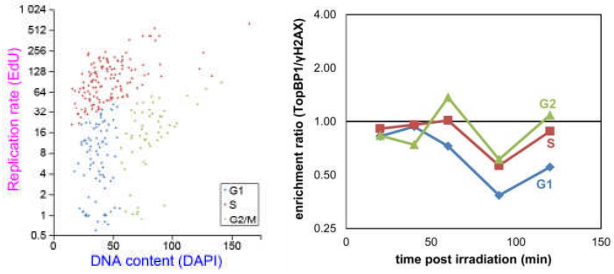

Figure 2. hiMAC-based cell cycle profile post 2 h (left). Ratio of the enrichment of TopBP1 and γH2AX in DNA damage tracks (right).

References

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