CELL-CYCLE SPECIFIC ANALYSIS AFTER ION MICROIRRADIATION AT SNAKE

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Abstract

Live-cell imaging (LCI) of irradiated cells allows observation of the temporal development of cellular response mechanisms, such as recruitment of proteins to the damaged site. For analysis of the influence of cell-cycle phase on the damage response mechanisms, live-cell markers of cell-cycle position are required. We developed a series of cell lines expressing cell-cycle markers and DNA damage response (DDR)-proteins tagged with different fluorescent proteins for LCI analysis after irradiation at SNAKE.

INTRODUCTION

The main method for LCI of cellular structures involves transient or stable transfection of cells with vectors encoding the gene for the protein to be observed tagged with a fluorescent protein, such as GFP (green fluorescent protein). Mostly in these vectors heterologous promoters are used, which leads to loss of the normal expression regulation (e.g. cell-cycle specific expression patterns) and overexpression of the tagged protein. In the case of cell-cycle markers, it is important to ensure that the transgenes do not interfere with normal cell-cycle control mechanisms.

MATERIALS AND METHODS

Vectors for expression of MDC1-eGFP and MDC1-tagRFP were described [1,2]. A vector for expression of G2-phase marker ΔCyclin B1-eGFP was constructed according to an idea presented by [3]. Vectors for the expression of Cdt1-mKO2 (G1 and very early S phase) and Geminin-mAG (S and G2 phase) according to [4] were obtained by MBL. A vector for expression of PCNA-Chromobodies for visualization of endogeneous PCNA was obtained by Chromotec. Stably transfected U2OS cell lines expressing Cyclin B1-eGFP + MDC1-tagRFP, Geminin-mAG + MDC1-tagRFP, Cdt1-mKO2 + MDC1-eGFP, or tagRFP-PCNA-Chromobody + MDC1-eGFP, respectively, showed close to normal proliferation rates. Irradiation of stably transfected cells (HeLa and/or U2OS) with single 55 MeV carbon ions in line patterns (1 µm distance in x, 5 µm distance in y) and subsequent imaging was performed at ion microbeam SNAKE of the MLL [5].

RESULTS

Mdc1 Foci form quickly (within seconds) after irradiation at the sites of ion transversal. Cell-cycle markers allow positive identification of cells in the various phases, thus enabling us to determine phase-specific recruitment kinetics (work in progress). In addition, cell-cycle markers allow observation of cells progressing in the cell cycle (see Fig. 1). Here, in the right cell, which is not labelled by G1 marker Cdt1-mKO, the line pattern of the irradiation set-up is reflected by the pattern of MDC1 foci early after irradiation. Later (5 h) due to cell movements the pattern is not clearly detectable anymore, while after 12 h the line pattern is visible again, albeit at a different orientation. Note that in the majority of cells this high degree of cell mobility is not seen and foci patterns are quite stable over time. The left cell was irradiated while in mitosis and individual foci become visible after cytokinesis. Concomitantly, the cells start to express G1 marker Cdt1-mKO2.

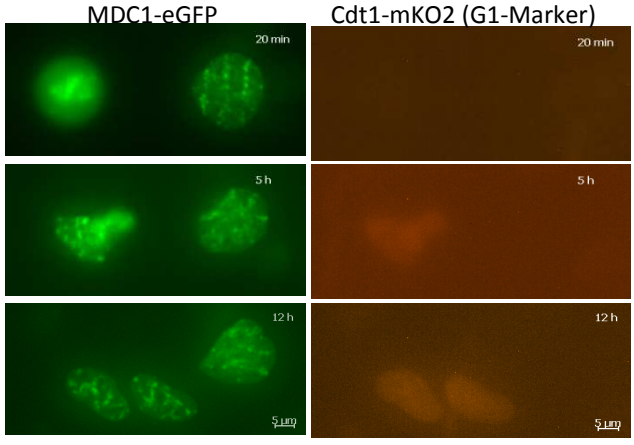

Figure 1: Mitotic (left) and non-G1 (right) cells irradiated with 55 MeV carbon ions in line pattern at SNAKE.

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