Cell Cycle Control and Apoptosis in HeLa Cells after Irradiation with SNAKE

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The ion microprobe SNAKE is an excellent tool for investigating radiobiological effects on a single cell level. Here we describe how to analyse cell cycle control and radiation induced apoptosis in HeLa cells (cervical cancer cell line) via immunofluorescene (IF) methods.

The cell cycle is a tightly regulated process taking place in every proliferating cell. Its main events are duplication of the genome (S(ynthesis)-phase) and division of the cell into genetically identical daughter cells (mitosis). These events are separated by gap-phases G1 (between mitosis and S-phase) and G2 (between S-phase and mitosis) in which cell metabolism and cell growth occur, as well as preparation for the two main events. A cell cycle control system ensures the correct duplication of the genome before the cell may enter mitosis. An important cellular response to radiation induced DNA damage is the arrest of the cell cycle at distinct checkpoints to prevent damage propagation. After successful DNA repair the cells proceed in the cycle. In case of repair failure the cells remain in arrest and/or undergo apoptosis. Apoptosis is a complex mechanism in which a "suicide" program is activated, leading to rapid cell death mediated by intracellular enzymes called caspases.

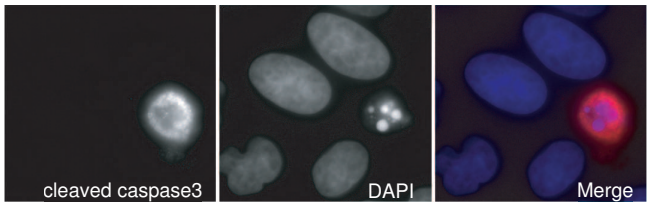

Fig. 1: IF detection of cleaved caspase3 (red) as a marker for apoptotic HeLa cells. DNA-staining with DAPI (blue) reveals typical chromatin structure in late apoptosis.

After irradiation with SNAKE the cells can be cultured for several days until fixation for immunofluorescence detection of cell cycle phase (fig. 2) and occurrence of apoptosis (fig. 1) in individual cells. DNA damage-induced cell cycle arrest leads to accumulation of cells in G2-phase, which can be visualized by staining the G2-phase markers CyclinB1 and CenP-F (Centromer Protein-F). Apoptotic cells are determined by detection of the active enzyme caspase3 (cleaved caspase3). We currently investigate the influence of different beam qualities (microirradiation with 55 MeV carbon ions, 20 MeV protons) on cell cycle arrest and apoptosis. Furthermore, effects of pulsed versus continuous 20 MeV proton beams are investigated whithin the excellence cluster MAP (Munich-Centre for Advanced Photonics).

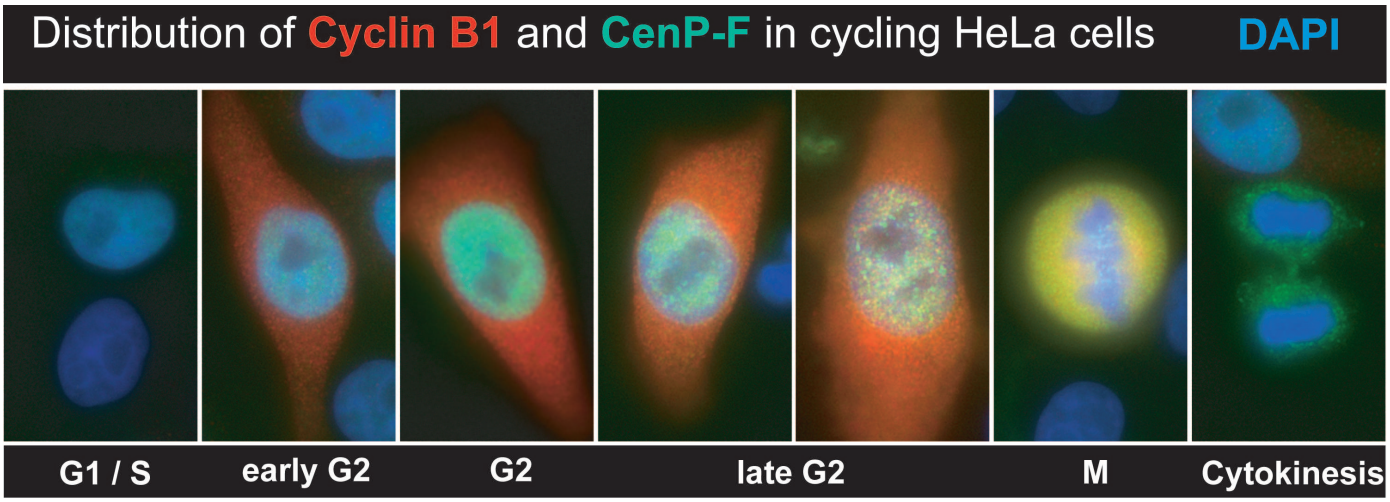

Fig. 2: Typical IF-signals of cell cycle markers CyclinB1 and CenP-F in HeLa cells during the cell cycle. CyclinB1 (red) is characteristic for cells in G2-phase and Mitosis (M), CenP-F (green) is present during S- and G2-phase and Mitosis. DAPI (blue): DNA-counterstaining.