Cellular bystander response after exposure to high LET irradiation.

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In this study various cellular responses of non-targeted cells following heavy ion exposure of human fibroblasts were investigated. Heavy ions are an excellent tool to elucidate the impact of ionisation density on the occurrence of bystander effects. An improved understanding of bystander responses is important with respect to risk estimation for accidental or therapeutical radiation exposure.

Human fibroblasts were exposed to low fluences of heavy ions (C, Ar and U with LETs in the range of 170 to 15000 keV/μm), traversing only a few cells by a particle. For selected endpoints, targeted irradiation of single cells was performed using a heavy ion microbeam. A medium transfer technique was applied to study the transmission of signals limited to soluble factors. At several time intervals after exposure, the cell cycle progression (FACS), the expression of CDKN1A and other cycle regulators (Western blot, immuno-fluorescence) and the amount of intracellular reactive oxygen species (ROS; DCF fluorescence) were assessed. In addition, the frequencies of sister chromatid exchanges (SCE) and the number of cells containing micronuclei (MN) were determined 3 days after exposure as indicators for changes or damage on chromosomal level in bystander cells. An overall induction of CDKN1A, but no distinct clusters of cells bearing an elevated expression level in the direct neighbourhood of the hit cells were observed several hours after exposure. This effect was accompanied by a transient delay in the initial G1 phase after exposure. The question was addressed whether the cell cycle related changes are linked to elevated levels of ROS, SCEs and MN in the bystander cells. Slightly enhanced frequencies of SCEs were detected for low fluencies of carbon ions. However, no pronounced bystander effect was observed with respect to the occurrence of micronuclei for carbon and uranium ions. The analysis of the intracellular levels of ROS revealed a small increase during the first hours after irradiation, occurring simultaneously to the transient induction of CDKN1A protein. During the following days, the levels of ROS remained unchanged.

Taken together, the exposure of a few cells to heavy ions with LET values covering a range of two magnitudes revealed a bystander effect with respect to transient, cell-cycle-related effects. We hypothesize that ROS may be involved. Effects giving an indication of DNA damage on the chromosomal level were not observed in bystander cells.