SUMMARY

Presented work is engaged with focused on molecular mechanisms activated by ionising radiation leading to reparation of DNA lesion or to induction of apoptosis. We used human leukaemic cell lines differing in expression of the tumor suppressor protein p53 (positive in T-lymphocyte MOLT-4 and negative in promyelocyte HL-60 cell lines, resp.).

In MOLT-4 cells we proved ATM kinase-dependent signaling pathway, which activates number of substrates involved not only in cell-cycle arrest but also in DNA reparation, to be functional. An out breaking finding is that DNA reparation is not effective in these cells, presumably due to an impaired phosphorylation of Nbs1 protein, an important part of reparation complex MRN. We assume this as one of the reasons for higher radio-sensitivity of these cells.

Radiation-induced activation of ATM kinase leads to rapid phosphorylation of checkpoint kinase-2 and to up-regulation and phosphorylation of p53 and its negative regulator - oncoprotein Mdm2.

Besides DNA reparation indispensable for further proliferation of the cells we focused on cell death. In each cell line studied we confirmed activation of both receptor and mitochondrial pathways of induction of apoptosis. In both of them the amount of antiapoptotic protein Mcl-1 increased after sublethal but not lethal doses. Later (with onset of apoptosis) it declined. Irradiation also induced cleavage of proapoptotic protein Bid and release of cytochrome c from mitochondria. An interesting finding is that in p53-positive conditions (MOLT-4) are both initiation caspases-8 and -9 activated simultaneously, while in p53-negative conditions is caspase-9 activation obviously delayed. This suggests that caspase-8 plays more important role in the cells lacking p53. Both of the cell lines also differ in the mode of apoptosis. The MOLT-4 cells exhibit wide disparity in the time of induction of apoptosis and they die mostly by so-called mitotic apoptosis. On the other hand, HL-60 cells undergo so-called delayed apoptosis (induced after G2 arrest necessary for DNA repair) after the low dose, or they die by rapid apoptosis induced by supra/lethal doses within hours after irradiation.

We proved phosphorylation of p53 on Ser^{15} to be dose-dependent up to 3 Gy (confirmed by Western blotting and ELISA) and therefore we proposed this protein as a biodosimetric marker of absorbed dose of ionising radiation. Using ATM/Chk-2/p53 signaling pathway, we also proved that caffeine (2 mM) increases cytotoxicity of low doses of radiation and can be exploited in combined therapy of cancer.