Role of cytoplasmic and nuclear signaling in apoptotic death of lymphocytes under X-irradiation

T. Andriichuk\textsuperscript{a}, S. Andreychenko\textsuperscript{b}, N. Raksha\textsuperscript{a} and A. Klepko\textsuperscript{b}

\textsuperscript{a} Taras Shevchenko National University of Kyiv, 64 Volodymyrska Str., Biological Faculty, 01033 Kyiv, Ukraine; \textsuperscript{b} SE 'Scientific Center for Radiation Medicine', 53 Mechnikova Str., 04050 Kyiv, Ukraine

kallav@mail.ru

The research aimed in studying a set of molecular events that are responsible for the induction of apoptosis in immunocompetent cells under X-irradiation. Rats were X-irradiated in the dose of 1 Gy with dose input 0,17 Gy/min. Afterwards rat splenocytes were isolated by the aid of Ficoll gradient. Detection of apoptotic cells was performed on flow cytometer PAS (Partec, Germany) using phosphatidyserine binding protein Annexin V conjugated to FITC in combination with vital dye propidium iodide. Single (SSB) and double strand (DSB) breaks in DNA were quantified by modified TUNEL method. Nuclear transcription activating factor (NF-κB) was measured in splenocytes nuclei by ELISE Kit (Oxford Biomedical Research). Enzymatic activities of caspase 2 and 8 were determined using BioSource kit. It has been shown that X-rays induced two fraction raise in apoptotic cells in the span of 3h after irradiation. By the end of this time interval the amount of DSB in nuclear DNA was shown to increase 3 fold that resulted in the evident elevation of activated NF-κB which migrated to the splenocytes nuclei. Simultaneously, the caspase 2 and 8 activities rose in 0,5h and then significantly decreased in 3h comparing to unirradiated control. The caspases in question are known to play an essential role in genotoxic stress-induced apoptosis. The data received are to be useful for further elucidation of apoptosis mechanisms in different immunocompetent cells.