

Assessment of the nuclear function of caspases following genotoxic stress

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Apoptosis is the best-known mode of programmed cell death that plays a crucial role in the regulation of embryogenesis and tissue homeostasis. Induction of apoptosis upon the administration of DNA-damaging agents is currently the most widely exploited therapeutic approach in anticancer treatment.

Caspases are the key apoptotic proteins that guide both the initiation and execution of apoptosis. Normally, caspases are present in the cytoplasm. Upon apoptosis induction, initiator caspases, and then, executioner caspases, become active and coordinate the dismantling of cellular compartments, including the nucleus [1]. Notably, in response to a range of apoptotic stimuli, nuclear translocation of apoptotic caspases has been reported. Yet, the molecular mechanisms underlying the event and the subsequent outcome remain unclear. The investigation of these mechanisms constitutes an important fundamental issue and might lay the foundations for the development of novel anticancer therapies.

To assess the nuclear events which take place in the course of apoptosis induction, a novel fractionation protocol has been developed. The protocol allows the isolation of purified nuclei from human cancer cells HeLa and Caov-4 and applies phase-contrast and fluorescence microscopy techniques, along with western blot analysis of subcellular marker proteins, for the validation of the integrity and purity of the obtained nuclear fractions. The developed approach in combination with western blotting and caspase activity assays has been employed for the assessment of caspase-2, -3, -8 and -9 redistribution following DNA damage. Nuclear accumulation of the active forms of the aforementioned caspases in response to treatment with a genotoxic agent (cisplatin) has been observed. The analysis of caspase-2 localization by confocal microscopy has confirmed the results obtained by western blotting and caspase activity measurement approaches.

Taken together, in the course of this work, the protocol allowing to follow the nuclear events occurring upon cell death induction and defining the cellular decision between life and death has been developed.

Источники и литература

- 1) Prokhorova EA, Zamaraev AV, Kopeina GS, Zhivotovsky B, Lavrik IN. Role of the nucleus in apoptosis: signaling and execution. *Cell Mol Life Sci.* 2015; 72(23):4593-612.