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Initiator and effector caspases simultaneously translocate to the nucleus during apoptosis

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Apoptosis is the best-known mode of programmed cell death that is crucial for organism development and homeostasis. Induction of apoptosis upon the administration of DNA-damaging agents is currently the most widely exploited approach in anticancer therapy.

The key role in apoptosis belongs to caspases. According to the conventional view, effector caspase-3 is the major protease that contributes to the disintegration of the nucleus during apoptosis. At the same time, the cleavage of a number of nuclear proteins by initiator caspases was reported. However, the nuclear translocation of initiator caspases during apoptosis remains an open question. In particular, given the especially contradictory data on caspase-2 localization and function, we were interested in its redistribution. However, to obtain a more complete picture, we analyzed the changes in the localization of both initiator and effector caspases, as well as of several other pro-apoptotic proteins.

To assess the nuclear events, which take place in the course of apoptosis, first we set up a subcellular fractionation protocol. The purity of the obtained fractions was validated by microscopy and Western blotting. DNA-damaging agent cisplatin was used as an apoptosis inducer. Treatment with cisplatin caused nuclear condensation, and then, fragmentation, which are both the essential steps and hallmarks of apoptosis. In response to cisplatin treatment of HeLa, Caov-4 (cervical and ovarian cancer with inactivated p53, respectively), MCF-7 (breast cancer, caspase-3 deficient) cells, specific accumulation of the catalytically active initiator caspase-2, -8 and -9 and effector caspase-3 in the nucleus was observed using Western blotting, caspase activity measurement and confocal microscopy. The translocation events were p53- and caspase-3-independent and occurred early during apoptosis, preceding nuclear lamina degradation and subsequent nuclear disintegration.

Although no distinct pattern was observed in the redistribution of caspase-2 during cisplatin-induced apoptosis, we also investigated the role of caspase-2 ubiquitination in the regulation of its localization and nuclear function by (co)-immunoprecipitation and mass spectrometry. Additionally, knockdown and CRISPR/Cas9-generated knockout of caspase-2 in Caov-4 cells resulted in a marked decrease in apoptosis upon genotoxic stress shown by flow cytometry, Western blotting and caspase activity measurement. Moreover, the cleavage of nuclear protein PARP1 that usually takes place during apoptosis was inhibited, supposing that caspase-2 might also play a role in its cleavage in the nucleus in response to DNA damage.

Overall, along with effector caspases, initiator caspases translocate to the nucleus during apoptosis, and via the cleavage of certain nuclear substrates, might promote the disintegration of the nucleus and apoptotic cell death.