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Ultra-structural and X-ray micro analysis of apoptotic cells U-937

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Cell culture U-937 is a convenient model for studying the "extinction" of cellular functions in experiment because these cells can be easily transformed in apoptotic state with different agents. Simultaneous study of functional and morphological changes in cultured cells treated with hypertonic shock and etoposide are revealing disturbances in ion homeostasis in the cells and structural rearrangements in intracellular organelles, such as mitochondria (M), endoplasmic reticulum (ER), Golgi apparatus (GA), cytoskeletal elements, and the ubiquitin-proteasome complex. The results obtained on ultra-structural changes in U-937 cells in the apoptotic state are largely consistent with analogous data available in the literature. One of the important characteristics of apoptotic cells on different stages of apoptosis is the presence of aggregates of thin osmiophilic particles in the nucleus and the cytoplasm. The aggregates are not limited by membranes and have varying density and size. Small rod-shaped particles approximately 12X30 nm in size are dominated in the aggregates. Immunocytochemical analysis of these cells with polyclonal Abs against $\alpha 7$ subunit of proteasomal protein and colloidal gold (10nm) has shown that these particles are proteasomes. Aggregates of osmiophilic particles have been described in literature in cells situated under extreme conditions: overheating, hypertension, hypoxia, reactive oxygen species, viruses, apoptosis state. Besides a spontaneous apoptosis associated with the differentiation of mammalian cells and tissues takes place in nature. For example, such processes are described during spermiogenesis and hibernation of animals. It is generally accepted that the segregates are formed under conditions of suppressed transcription in the nucleus and involved in the storage and degradation of various mRNA, RNPs and misfolded and overexpressed proteins that should be eliminated by proteolysis using the ubiquitin-proteasome system. In our work we have visualized with electron microscopy one component of this system—proteasomes. Their presence is an indirect confirmation of the involvement of protein ubiquitin in these processes, because these two components operate in tandem. The results of X-RMA of elemental composition of cells obtained in this study are consistent with the results of other studies. Despite the considerable number of studies devoted to the analysis of changes in the intracellular ions during apoptosis, we have revealed changes in the intracellular contents of Na⁺ and K⁺ at the level of single cells during apoptosis induced by osmotic shock. An increased ratio of intracellular Na⁺/K⁺ compared to the control for the majority of cells in apoptosis is obtained.

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