DEFINING THE ROLE OF CYTOSKELETAL COMPONENTS IN THE FORMATION OF APOPTOPODIA AND APOPTOTIC BODIES DURING APOPTOSIS (FINAL ESSAY)

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Cell Biology (BIOL 293)

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April 24, 2022

Apoptosis, also called “programed cell death” is a process where the cell regulated its own death through the production of certain enzymes. These enzymes cause the degradation of nuclear and cytoplasmic material, and the cell then breaks into fragments known as apoptotic bodies. The apoptotic bodies are then consumed by phagocytes.

Apoptosis can be caused by factors such as infections, misfolding of proteins due to mutations, radiation, hypoxia, and free radicals. Apoptosis is also used as a homeostatic mechanism where cells that are not needed are eliminated, which helps maintain a steady population of cells.

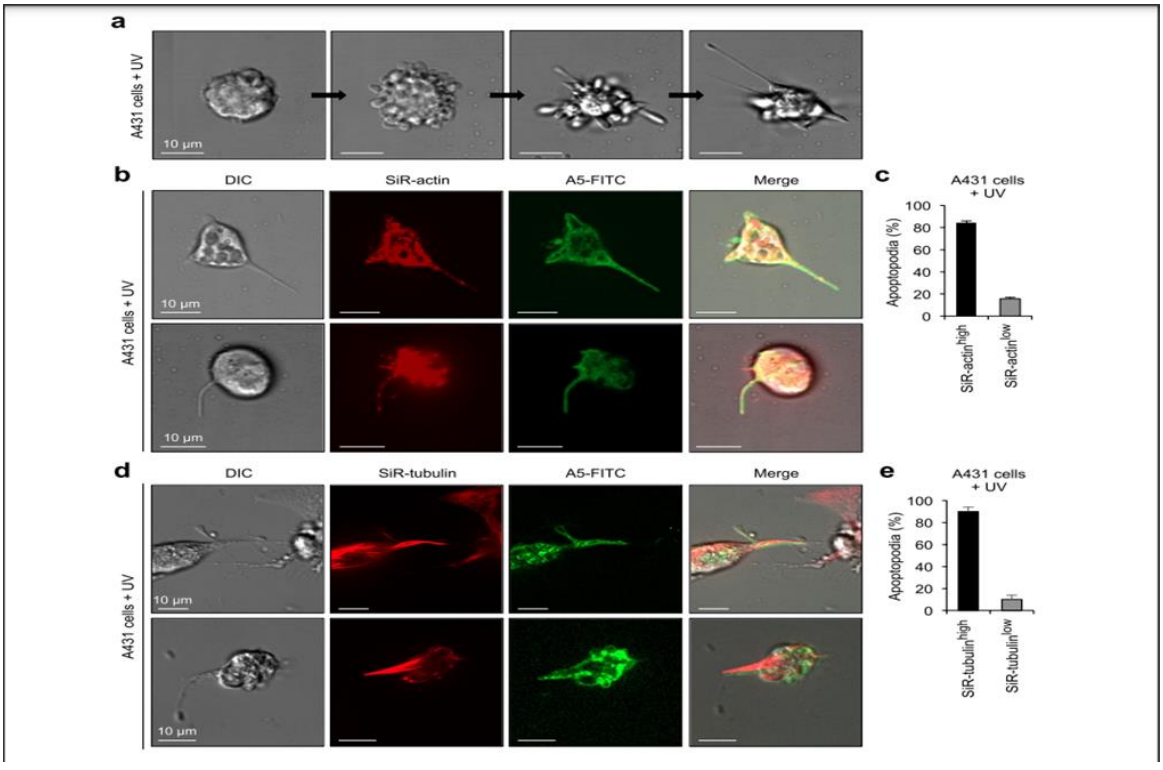
During apoptosis the cell undergoes morphological changes. The cell shrinks becoming smaller in size, the cytoplasm and organelles become tightly packed the nuclear chromatin shrinks and becomes condensed at the center of the cell, this process is known as pyknosis. After this process the chromatin material will undergo karyorrhexis, which means the nucleus of the cell degrades and becomes fragmented. After karyorrhexis the cell starts to form membrane blebs on its surface and starts to break off into smaller fragments which are called apoptotic bodies. The apoptotic bodies have portions of the cytoplasm, organelles, and nuclear fragments of the cell.

A cell can undergo apoptosis due to several reasons and depending on etiologic factors, apoptosis can be initiated by one of two different pathways (Elmore, 2007). Apoptosis can be initiated by signals from the intrinsic and extrinsic pathways. With the intrinsic pathway mitochondria become leaky and ooze out proteins called cytochrome C which initiates apoptosis. Usually, the cytoplasm and mitochondrial membrane have proteins called Bcl-2 and Bcl-x which are anti-apoptotic and will preserve the integrity of the mitochondrial membrane, preventing apoptotic proteins like cytochrome C from leaking into the cytoplasm. But if there is an absence in growth signal, or insults due to radiation or protein misfolding, stress proteins called “BH3 only” proteins are stimulated. These BH3 proteins are composed of BIM, BID, and BAD proteins which block the function of Bcl-2 and Bcl-x (Ren et al., 2010). These proteins will further activate two pro-apoptotic effectors called BAX and BAK, which create channels in the mitochondrial membrane, allowing intra-mitochondrial proteins like cytochrome C to leak into the cytoplasm (Ren et al., 2010). Once cytochrome C is in the cytoplasm, it binds with a protein called apoptosis activating factor 1 or Apaf-1 for short. When cytochrome C binds with Apaf-1 it forms a complex called apoptosome. This complex will then bind with caspase 9 which will cleave and activate adjacent caspase 9 molecules. Caspase 9 is an initiator and activates executioner caspase which leads to apoptosis. Cytotoxic T lymphocytes also known as CD8 T cells cause apoptosis of infected cells or tumor cells by Fas ligand and Fas receptor interactions (Farhood, Najafi, and Mortezaee, 2019). This attraction will cause apoptotic signals leading to apoptosis.

Apoptotic bodies are coated with a phospholipid called phosphatidylserine, which is recognized by phagocyte receptors. Apoptotic bodies can also be coated with opsonin’s which are also recognized by phagocytes which will facilitate rapid phagocytosis of apoptotic bodies.

The cytoskeleton of eukaryotic cells is made of filamentous proteins, and it provides mechanical support to the cell and its cytoplasmic constituents. All cytoskeletons consist of three major classes of elements that differ in size and in protein composition. Microtubules are the largest type of filament, and they are composed of a protein called tubulin. Actin filaments are the smallest type, and they are made of a protein called actin. Intermediate filaments, as their name suggests, are mid-sized. Unlike actin filaments and microtubules, intermediate filaments are constructed from several different subunit proteins (O'Connor, 2014). Microtubules (tubulin) provide the basic organization of the cytoplasm within the cell and actin filaments support and strengthen the plasma membrane (O'Connor, 2014).

The researchers wanted to determine if actin and tubulin cytoskeletal components were involved in the formation of apoptopodia. The data collected from the research demonstrate apoptopodia as a novel type of membrane protrusion that could be formed in the absence of actin polymerisation and microtubule assembly (Caruso, S., Atkin-Smith, G.K., and Baxter, A.A. et al. 2019). The methods used in this research involved cell culture, isolation of primary mouse monocytes and thymocytes, 3D culture imaging, flow cytometry, CRISPR/Cas9 gene editing, statistical analysis, ATP release assay, immunoblotting, apoptotic protrusion velocity quantification, DIC and fluorescence microscopy, and induction of apoptosis which was done by UV irradiation.



This is figure 6 from the research and it goes over localization of F-actin and microtubules during apoptotic A431 cell disassembly. **a** Representative time-lapse DIC microscopy images of UV-irradiated A431 epithelial cells undergoing apoptotic cell disassembly over 4–5 h. **b** Representative confocal microscopy images of UV-irradiated A431 cells stained with SiR-actin

(red) and A5-FITC (green). **c** Quantification of the percentage of apoptotic A431 cells with protrusions containing SiR-actin staining. **d** Representative confocal microscopy images of UV-irradiated A431 cells stained with SiR-tubulin (red) and A5-FITC (green). **e** Quantification of apoptotic A431 cells with protrusions containing SiR-tubulin staining. Error bars represent SEM (n=3), data are representative of three independent experiments. Apoptosis was induced by 150 mJ/cm2 UV irradiation (Caruso, S., Atkin-Smith, G.K., and Baxter, A.A. et al. 2019).

The researcher's goal with this study was to determine if actin and tubulin cytoskeletal components were involved in the formation of apoptopodia. As described in this study, F-actin was found in most apoptopodia even when actin polymerization was targeted pharmacologically, suggesting that F-actin may have other functions within apoptopodia, possibly stabilizing the protrusion or parts of it for a period of time (Caruso, S., Atkin-Smith, G.K., and Baxter, A.A. et al. 2019). The results of this study showed that the apoptotic membrane protrusions represent a unique class of membrane protrusions which are generated in the absence of certain cytoskeletal components. This research went into a lot more detail on apoptosis and different things that cause it then what was talked about in class which makes sense since it was a paper completely on apoptosis, but the general idea of apoptosis stayed consent in this paper and in class.

Apoptosis or the programed death of cells is a process where the cell regulated its own death through the production of certain enzymes. Better understanding this process will only benefit the scientific field and the research in this paper did precisely that and the methods used in the research and the results from this research can be used in future research to better understand the processes and apoptosis and what causes these different processes.

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