Scientific Literacy Essay

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Apoptosis can best be described as the scheduled and organized death of cells, resulting in the formation of apoptotic bodies, or ApoBDs. It eliminates cells that are unwanted during early development. Christopher P. Austin, M. D., described cells that neglect to be eliminated as turning into cancer cells (Austin, n.d.). When too many cells neglect to go through apoptosis, it results in many neurodegenerative diseases in healthy, young cells. Apoptosis is different from necrosis, which is when cells die due to trauma or lack of nutrients (Minikel, 2013). Apoptosis goes through the following steps: formation of membrane blebs, protrusion formation, and finally cell fragmentation (Caruso, S., 2019).

Although each step in this process is equally as important as the other, it is the second step, protrusion formation, is the step that is mainly focused on for the paper in the question of this essay. The paper, published in 2019, discussed the role of cytoskeletal components when forming apoptotic bodies and other "apoptodia" (Caruso, S., et. al., 2019). It described both the presence and role of F-actin and microtubules in apoptodia that is generated by T-cells and monocytes. T-cells are a type of white blood cell that is a part of the immune system and develop from stem cells in bone marrow (National Cancer Institute, n.d.). Monocytes are types of immune cells that are made from bone marrow that travel through the blood to tissues within the body. These monocytes then become macrophages which surround and kill microorganisms (National Cancer Institute, n.d.).

After the induction of apoptosis, a T-cell or monocyte undergoes many morphological changes, starting with the "bulging/blebbing" of cells, where T-cells start to look like an object with an unclear shape with many rounded protrusions from it. These protrusions from the cell

are called “membrane blebs”. A monocyte, on the other hand, still mostly resembles the image of a spherical cell, with only a few rounded protrusions from it. The cells then undergo a second morphological change with the creation of “apoptopodia”, or cellular machinery that are outside the cell during the process of apoptosis. T-cells generate apoptopodia that resemble smaller cells with a “connection” between them and the main/host cell. Monocytes, however, generated what can be described as “beaded” apoptopodia, in the way that the host cell has “beads” of apoptopodia leading away in branches from the host cell.

Finally, the last major morphological change is basically just the cell’s apoptopodia diverging from the host cell. For T-cells, this is just the cell releasing those bonds between it and the apoptopodia, and the larger apoptopodia disassembling into apoptodic bodies and other usable material for the next cell. For monocytes, the process more closely resembles the many small apoptopodia turning into apoptodic bodies by “spewing” away from the host shell like a shotgun’s blast. The following picture was taken from the essay in question, and visually describes the morphological changes completed during the host cell in apoptosis.

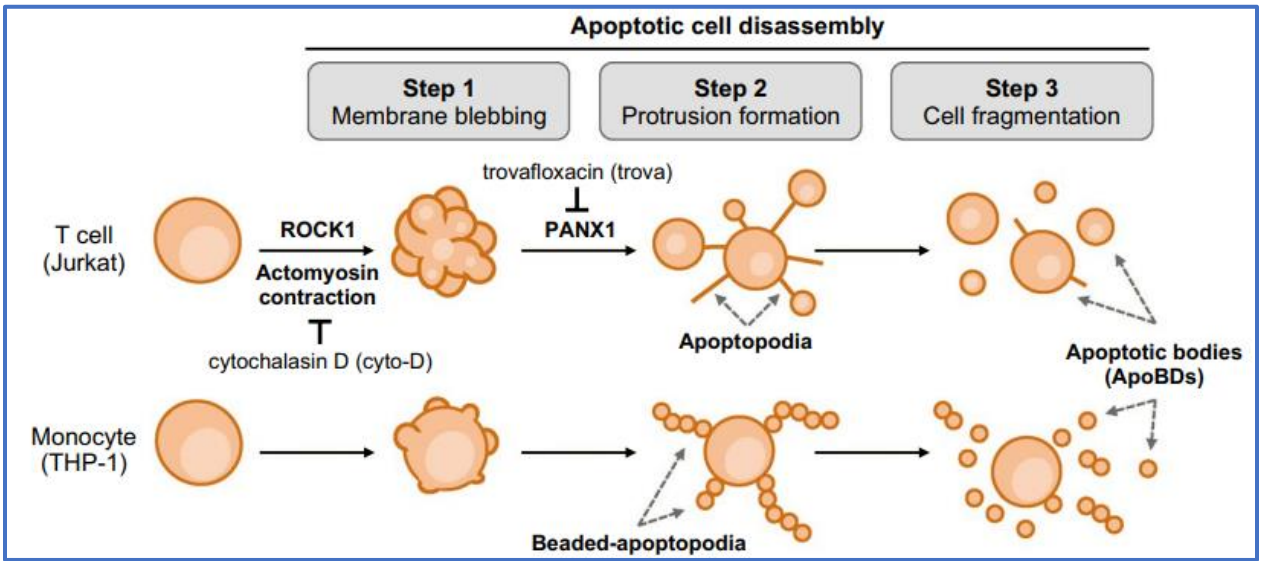

Figure 1: A Visual Representation of Apoptotic Cell Disassembly

Two very important cytoskeletal components of apoptosis in T-cells and Monocytes are called Actin and Tubulin. Actin, or in this case filamentous actin/F-actin, is cleaved by a protease enzyme called caspase during apoptosis, creating t-Actin and Fractin. This t-Actin induces the morphological changes described above (Ren, W., et. al., 2021). Tubulin, on the other hand, is used to regulate apoptosis by binding to death receptor 5, which, when activated, induces apoptosis (Twomey, J., et. al., 2018). It also polymerizes into long chains that form microtubules, which serve as a skeletal system for living cells (Yarris, L., 1998).

The article being in question suggests that both Tubulin and F-actin are involved not only in the beginning steps of apoptosis with the formation of membrane blebs but also in the formation of apoptotic protrusions and apoptodic bodies.

Major F-actin Findings

The paper in question sought to find the role of F-actin in apoptotic membrane protrusion formation. They utilized a cell-permeable probe called SiR-actin to monitor F-actin localization. They also stained the plasma membrane using either annexin A5 or anti-CD45 to visualize the apoptopodia. They first monitored apoptotic Jurkat T cells after inducing apoptosis using UV light. They found that both F-actin-rich and depleted protrusions were present and could be observed. Approximately 90% of the apoptopodia-forming cells produced F-actin-rich apoptopodia. They replicated this experiment similarly with monocytes and found that 55% of the apoptopodia-forming cells produced protrusions containing F-actin at all.

To find out whether actin polymerization contributed to the formation of the apoptopodia, they treated Jurkat T cells with cytochalasin D to stop actin polymerization while apoptosis is being monitored. After the induction of apoptosis, cytochalasin D treatment had no

effect on the amount/percentage of cells that formed apoptopodia. They concluded that with these results, apoptopodia does indeed contain F-actin, but F-actin polymerization is not required for the formation of apoptotic membrane protrusions.

Major Microtubules Findings

The study's second goal was to seek out the role of microtubules in apoptotic membrane protrusion formation. As stated before, microtubules are a major cytoskeletal component, yet are suspected not to play a large role in the formation of apoptodic bodies and membrane protrusions. Like with the F-actin experimentation, Jurkat T cells and THP-1 monocytes were stained with cell-permeable probes (SiR-tubulin) to monitor how the microtubules distributed during the process of apoptotic cell disassembly. The apoptotic Jurkat T cells that generated apoptopodia formed both microtubule rich and depleted apoptopodia. It was shown by the data that 61% of the apoptopodia-forming cells were rich in microtubules. However, when the THP-1 monocytes were monitored and went through apoptosis, only 20% of all the apoptopodia-forming apoptotic cells generated microtubule-rich apoptopodia. They noted that apoptotic primary mouse thymocytes and monocytes generated apoptopodia-containing microtubules.

They next treated Jurkat T cells with noco (a microtubule destabilizing drug), or nocodazole directly after the start of apoptosis. They then monitored cell disassembly. It was found that noco had no effect on the percentage of apoptotic Jurkat T cells forming apoptopodia. However, in the THP-1 monocytes, the noco partially impaired apoptopodia formation. They noted that noco treatment reduced the percentage of microtubule-rich apoptopodia generated by Jurkat T cells and THP-1 monocytes, except less with the latter.

Other Findings

It was found that the disruption of F-actin polymerization, but the continued formation of microtubules inhibits apoptotic bodies. By inhibiting F-actin polymerization using either cyto-D or lantrunculin A, it was found that apoptotic bodies were not formed. This was hypothesized to be due to the blocking of membrane blebbing due to the dependence membrane blebbing has on actomyosin contraction. It was determined that F-actin is necessary for the apoptotic cell disassembly process, but not the formation of apoptotic bodies. The inhibition of microtubule assembly had no effect on the formation of apoptopodia or apoptosis as a process.

In the epithelial carcinoma cell line (A431 cells), the role of actin and tubulin in the disassembly of cells was determined. First, UV-irradiated A431 cells were observed and were found to be able to generate rigid apoptotic membrane protrusions. These protrusions were found to "shoot" out of the cell at an average velocity of 1.30 μm/min. The A431 cells were then stained with SiR-actin and SiR-tubulin and monitored concerning the distribution of F-actin and microtubules throughout apoptosis. Around 90% of the cells generated membrane protrusions rich in both F-actin and microtubules. The A431 cells showed the same results as the previous Jurkat T-cells when the inhibition of actin polymerization was introduced. However, when the inhibition of microtubule assembly was introduced, there was a stop in apoptotic membrane protrusion formation with minimal effect on total apoptotic body formation by the A431 cells. It was concluded that microtubule assembly is important for forming membrane protrusions but does not play a major role in the disassembly of apoptotic A431 cells.

Conclusion

The study in question sought to find the role of F-actin and Microtubules in the formation of apoptotic bodies and apoptopodia during the process of apoptosis. It was found that although F-actin polymerization plays a role in the formation of apoptotic bodies, it is not the driving force of apoptosis by any means, and is more of a catalyst, for lack of a better word. Microtubules were also shown not to be important in the creation of apoptotic bodies, whether the concentration of microtubules is rich or poor in the degrading cells. In the end, it was determined by the scientists that apoptopodia, when referenced by the membrane protrusions created during apoptosis, can be formed in the absence of both F-actin polymerization and microtubules assembly. Not only that, but apoptotic membrane protrusions or apoptopodia are a unique class of membrane protrusions that can be generated in the absence of cytoskeletal components, mainly F-actin and microtubules.

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