Jordan Medical Journal, Volume 56, No.1 2022
DOI: https://doi.org/10.35516/jmj.v56i1.232

An Ultrastructural Perspective on Cell Death

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Abstract

In the field of cell death, there is still a wide gap between the molecular models and their ultrastructural phenotypes. Because only very few published works included electron microscopy (EM) images, many ultrastructural features have not yet been incorporated into the descriptions of death modes. Some of the EM features that appear in dying cells have not been incorporated in describing death modes. It includes the accumulation of lipid droplets and glycogen, the appearance of extranuclear chromatin in the cytoplasm, and the various ways mitochondria become damaged. We argue that electron microscopy should be routinely included in these studies because it exposes some new features that molecular studies do not. It has successfully recognized new modes of cell death, such as entosis, methuosis, and paraptosis. Elucidating the precise sequence of events in death modes could be the cornerstone for offering the proper therapy of many diseases by slowing down or stopping the progression of degeneration. This review presents our own experience applying ultrastructural interpretations to death modes and explaining their biochemical implications. We complement the molecular and biochemical data and point out missing features that should be considered and studied.

Keywords: Apoptosis, mitophagoptosis, mitophagy, necrosis, necroptosis, pyroptosis, chromatin leakage, lipid droplets, glycogen, extranuclear DNA.

(J Med J 2022; Vol. 56 (1): 91- 114)

Introduction

The research community has mainly been biased towards apoptosis. Consequently, apoptosis was assumed to be the dominant mechanism involved in most cell death situations and, therefore, thought to be responsible for many physiological and pathophysiological conditions. However, as we now realize, regulated necrosis (or necroptosis) or unregulated mitophagoptosis may be the more prominent modes of cell death. Death can be diverse in its mechanisms since redundancy and pleiotropy are salient characteristics of life[1].

Cell death research has not yet matured. A consensus on classification and the scope of events is lacking, and a few observed death modes have not been adequately characterized or described. Along these lines, clinical, diagnostic, and therapeutic benefits can be gained from utilizing the underpinnings of various death types since they indicate pathological causes and pathways to degenerative diseases (such as age-related macular degeneration [AMD]). Understanding death mechanisms may yield insights for prevention and treatment programs. Unfortunately, cell death literature rarely incorporates ultrastructural data from electron microscopy (EM) or includes EM images to support their conclusions. Ultrastructural EM shows more types of cell non-apoptotic death than described in the literature, such as entosis, methuosis, and paraptosis[2]. As we will show in this review, EM is essential for cell death research to connect the ultrastructural

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phenotypes seen by EM with the molecular profiles.

Mitochondrial Contribution to Cell Death

The evolutionary origins of death mechanisms can be traced back to the prokaryotic bacteria, albeit in simpler forms such as the toxin-antitoxin systems[3]. Some α-proteobacteria contain metacaspases that are significant players in apoptosis. Since mitochondria are descendants of α-proteobacteria, they are probably the ancestral source of the metacaspases in the eukaryotic cells[4]. Although bacterial suicide could be a defense mechanism to trigger the release of more toxins, bacteria can continue to exist for an indefinite time without the inevitability of death. However, the end of the eukaryotic cell is inevitable, most likely because there are several elaborate death mechanisms[5]. As we propose, it is one vulnerability of the imperfect eukaryotic system. Another vulnerability is developing cancer.

There are many reasons why death is inevitable in eukaryotes, especially in multicellular organisms. Among these is the need for tissue morphogenesis, differentiation, and maintenance of homeostasis; all these could not occur otherwise[6]. Other reasons include eukaryotic cell senescence, infection by a pathogen, inflammation, hypoxia, dehydration, starvation…etc. Additionally, insults that cause cell membrane and genetic material alterations can induce cell death[7].

A healthy cell can undergo a temporary state of illness and, by reversing the changes, gets back to its healthy state. In contrast, irreversible changes due to mutations and permanently dysregulated expressions lead to cell death (Fig. 1). Almost 50% of the cell's energy is spent on maintenance and repair of its organelles and parts [8]; therefore, a dip in energy supply below 50% causes deterioration of the cell's components: chromosomal and chromatin packing, membranes integrity…etc. Mitochondrial dysfunction is central to the timing and severity of cellular deterioration[9]. Increased mtDNA mutations or diminished oxidative phosphorylation (OXPHOS) do not increase reactive oxygen species (ROS); however, the dysfunction of the respiratory chain causes premature aging in mtDNA mutator mice[10].

The amount of ATP in a cell could be the essential determining factor of the type of death pathway. The mitochondria play an essential role in the cell death mechanism that the cell will undergo: apoptosis, necroptosis, pyroptosis, or mitophagoptosis[8]. Apoptosis is an energy-intensive pathway, followed by mitophagoptosis, pyroptosis, and necroptosis. The latter is described as aborted apoptosis when the cell runs out of ATP before completing apoptosis.

The Significance of Lipid Droplets in Cell Fate

Publications that tie lipid alterations to various death processes fail to connect lipid droplets to cell death. Lipid droplets are triglycerides-packed membrane-less spheroidal bodies that appear grayish in EM images[11]. Cells may develop various lipid droplets in their cytoplasm, mostly in response to distress (including temporary hypoxia) or a permanent shift to a hypoxic phenotype. Lipid droplet homeostasis is regulated by hypoxia-inducible lipid droplet associated HILPDA[12]. On a biochemical level, the presence of lipid droplets could indicate the cell dependency on glycolysis as an energy source and a dysfunctional oxidative phosphorylation process[13]. Under hypoxic or pseudohypoxic conditions, glycolysis produces an excessive amount of pyruvate that is converted and stored as fatty acids in lipid droplets. Under normoxic conditions, pyruvate is converted to acetyl-CoA in the mitochondria; however, when the latter is not processed any further, it is used in the cytoplasm for lipid synthesis[13].

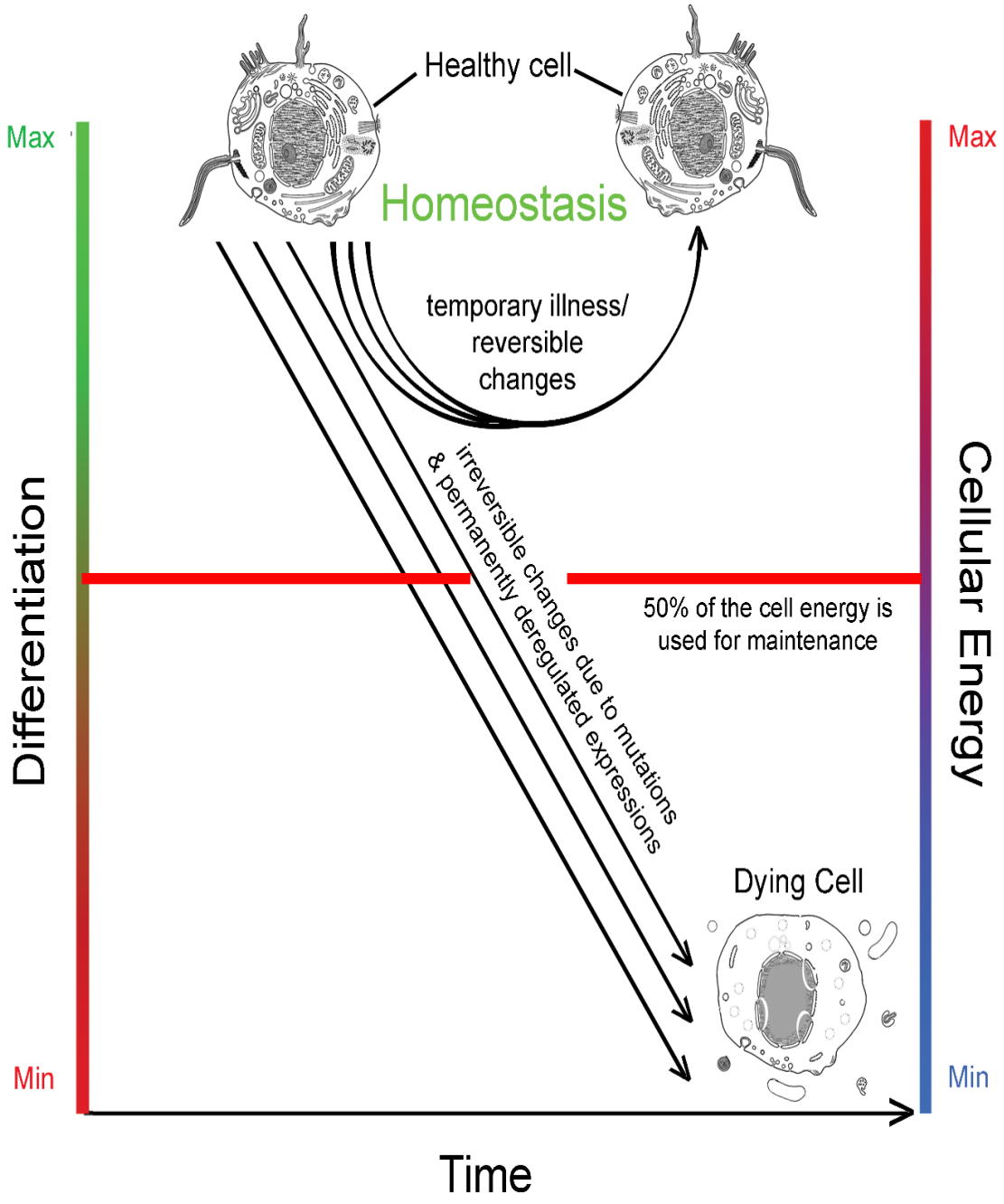


Figure 1.
The relationship of cellular energy, differentiation, and death. The dipping of energy production below the maintenance threshold triggers death signaling mechanisms such as apoptosis, or death without signaling such as necrosis.

Furthermore, such a condition indicates that oxidative phosphorylation is dysfunctional, and thus, nothing or little ATP is produced by the mitochondria. Suppose the formation of lipid droplets precedes death initiation, which we think it does in most cases. In that case, some mitochondrial dysfunction occurs before the initiation of the death process, such as apoptosis, necroptosis, or pyroptosis[9]. This points out the significance of detecting lipid droplets and related compounds such as pyruvate, lactate, and acetyl-CoA in the cytoplasm as indicators of pathology or impending cell death.

The Significance of Glycogen in Cell Fate

Although glycogen is usually stored in the liver and skeletal muscles, two organs occasionally undergo periodic hypoxia; glycogen in the cells of other tissues is usually a sign of distress. It could signal metabolic switching to a permanent hypoxic phenotype where glycolysis is the primary energy source[13]. Glycogen accumulation is easily visible by EM as black inclusions in rosette formation ranging from 4 to 10 nm[11]. As our EM images show, glycogen accumulation is abundant in pyroptosis (see Fig. 5F, glycogen appears as black dots). Glycogen synthase kinase-3 (GSK3), a key enzyme in glycogen synthesis, is overexpressed in several pathological conditions and is a promoter of apoptosis[14,15]. Unfortunately, the role of glycogen accumulation and its enzymes in cell death is missing in the literature.

Major Classes of Cell Death

Death types can be classified into two major classes (Fig. 2). The first class encompasses the deaths that involve death signaling, i.e., programmed cell deaths, including apoptosis, necroptosis, and pyroptosis. The second class comprises death types that do not involve death signals such as necrosis, mitophagoptosis, death by senescence, and chromatin leakage.

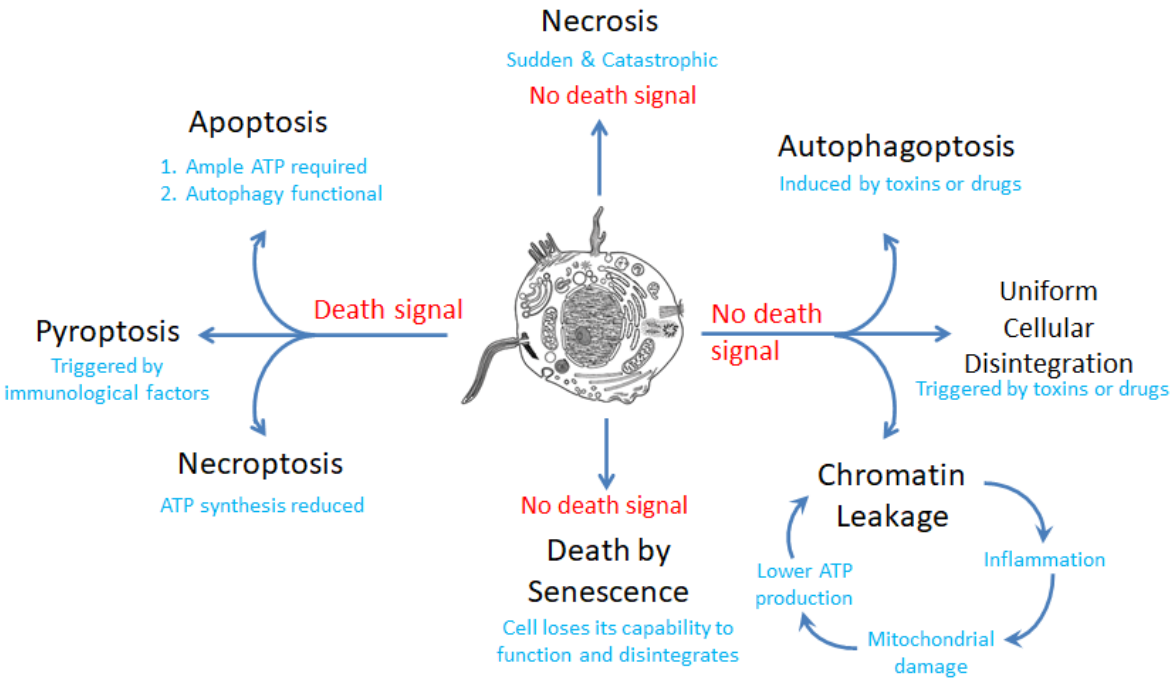


Figure 2.
Summary of major death types. There are two major classes of cell death, one involves a death signal such as apoptosis and pyroptosis, and the other does not involve any death signal such as necrosis and death by senescence.

First Class: Deaths Involving Death Signaling

1. Apoptosis

Apoptosis is energy-requiring programmed cell death. It results in the caspase-mediated breakdown of the cytoskeleton and chromatin. This death mechanism, unlike necrosis, is a clean process that does not leave any cellular debris and thus, does not illicit any inflammatory reaction (Fig. 3). Therefore, apoptosis is more suitable for tissue development and maintenance processes[6]. The internal digestion of the cell is contained within the cell membrane and causes the cell to shrink to a smaller dense vesicle that is termed the apoptotic body (Fig. 3D & E). The apoptotic body is eventually phagocytosed by a macrophage or microglial cell and digested or dumped into the intestines[16].

Morphological changes of apoptosis observed by TEM include cytoplasmic and nuclear shrinkage, pyknosis (the condensation of nuclear material), which is the hallmark of apoptosis, chromosomal fragmentation and karyolysis, blebbing of the cell membrane (more pronounced in tissue culture than in tissues), and disintegration of cellular organelles. This process dissolves the cell into a well-enclosed dense apoptotic body[2].

Furthermore, the biochemical reactions of apoptosis encompass the following: protein cleavage by caspases (cysteine proteases), the collapse of the cytoskeleton and nuclear matrix, protein cross-linking by transglutaminase, DNA breakdown in 50-300 kilobase (kb) pieces, phagocytic recognition by translocation of phosphatidylserine to the outer side of the cell membrane, and mitochondrial permeability and release of cytochrome C[5].

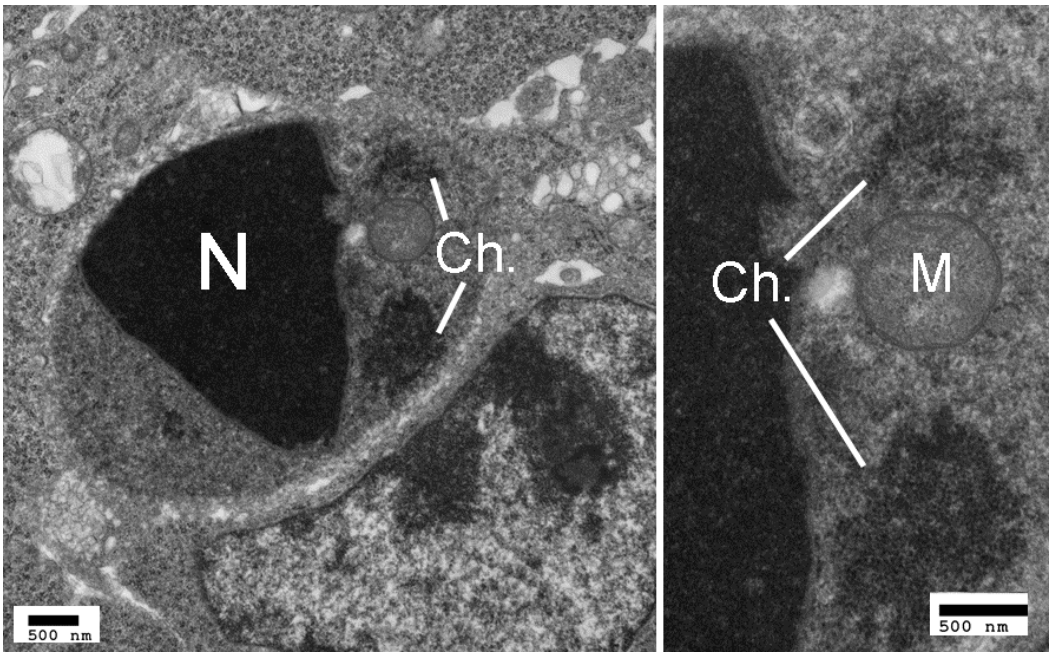
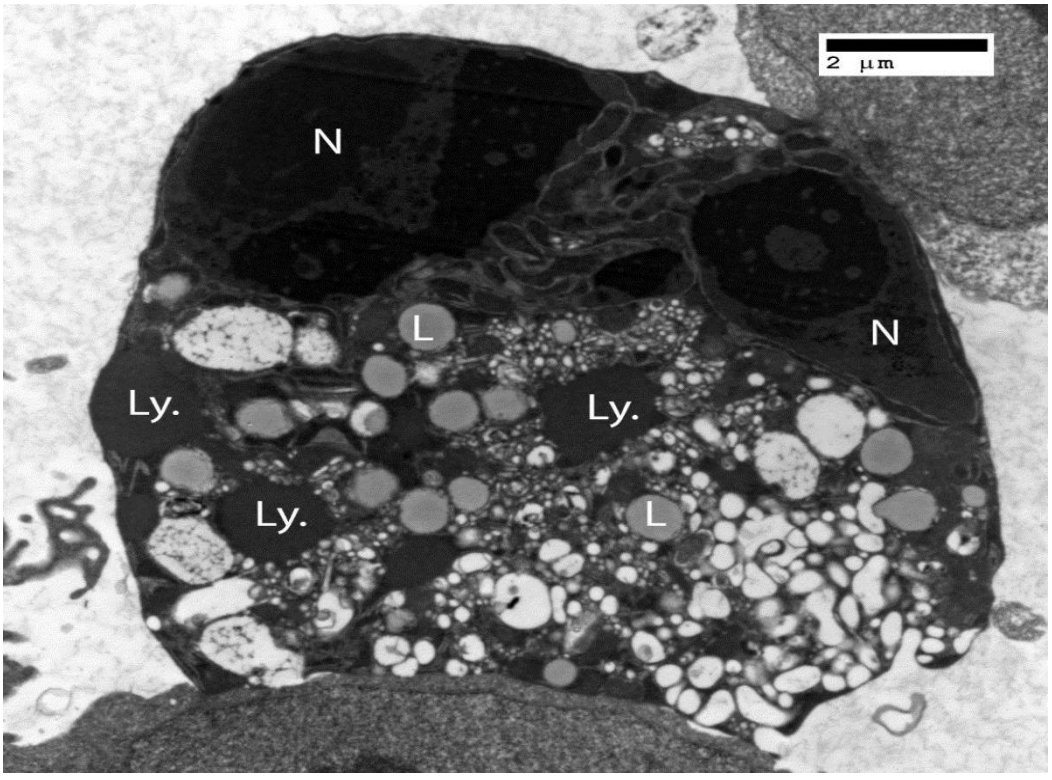


Figure 3. A.



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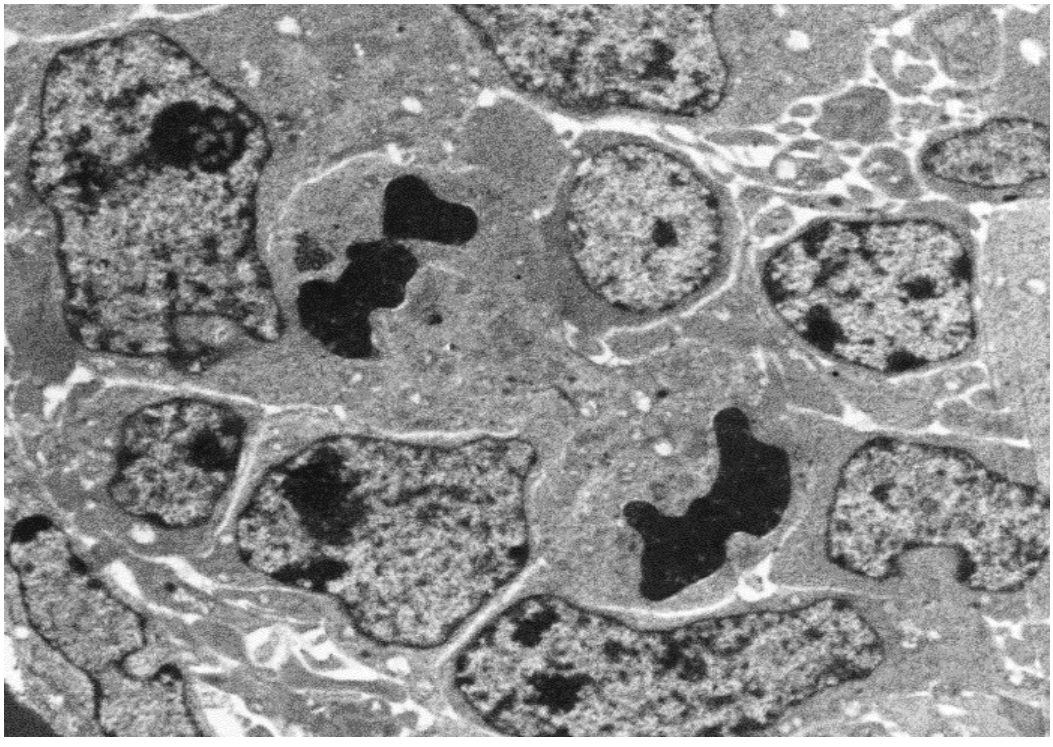

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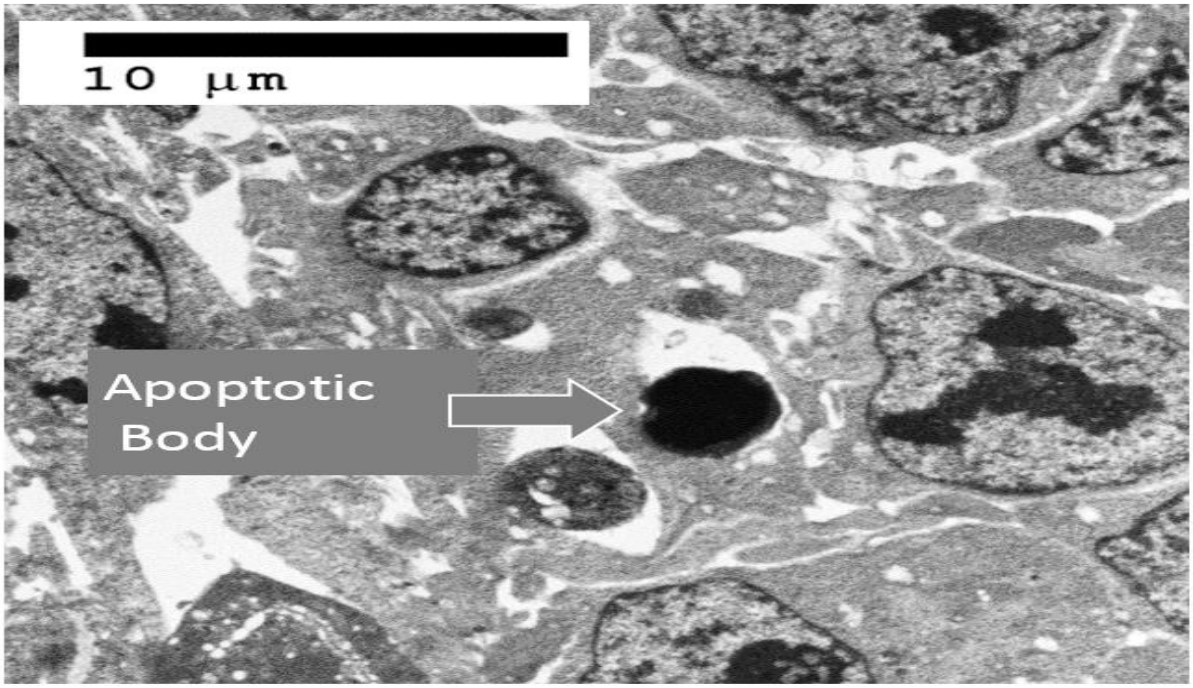

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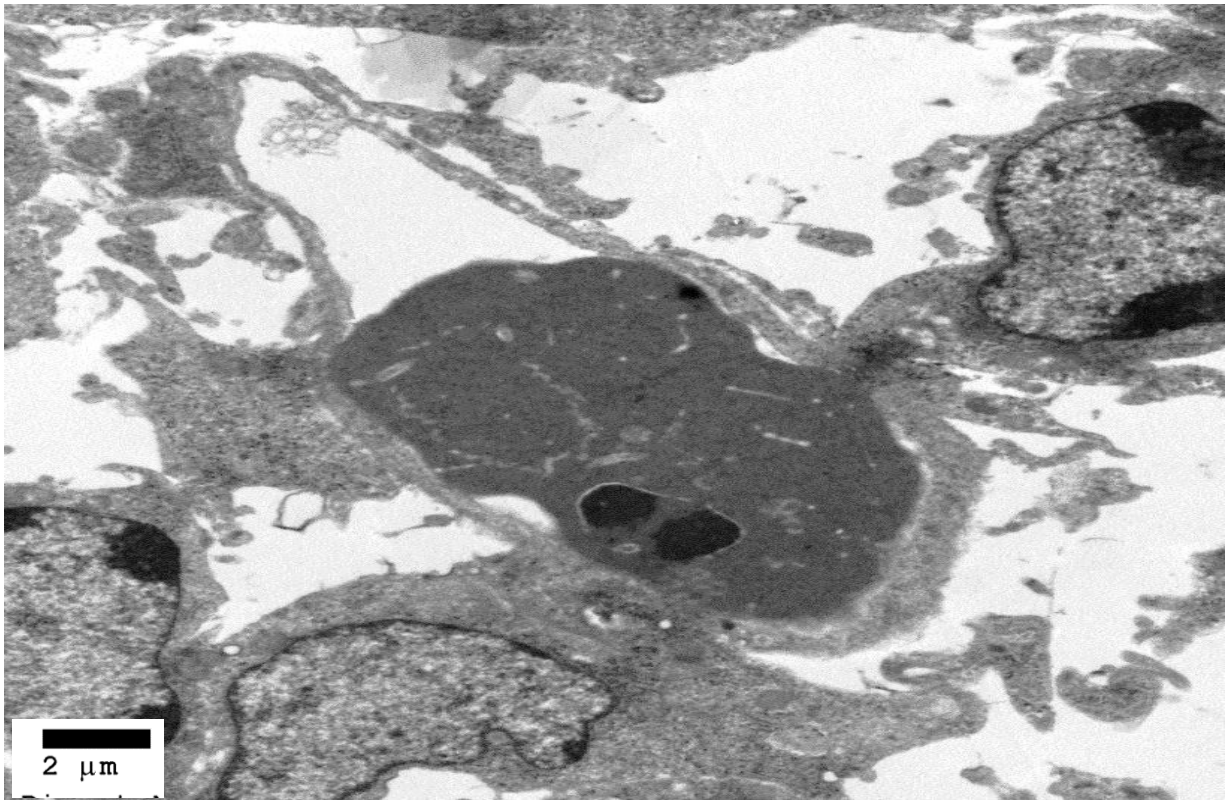

Figure 3. E.

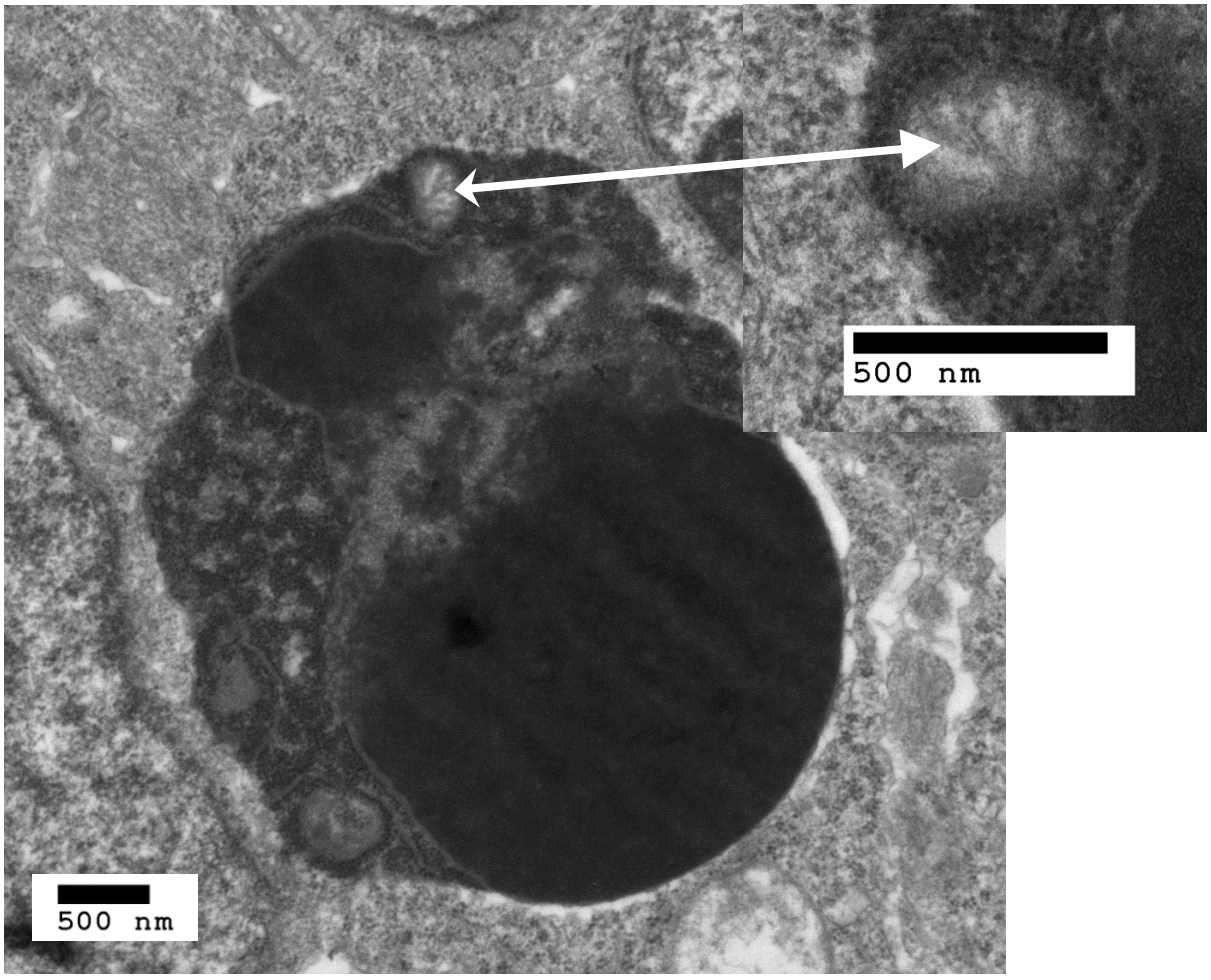

Figure 3. F.

Figure 3.

Various apoptotic cells at various stages. A. An apoptotic cell (in a developing mouse eye) showing shrunken dense nucleus (N), leaked chromatin (Ch.), and swollen degenerate mitochondrion (M), and intact plasma membrane. The leaked chromatin in the cytoplasm phenomenon is undescribed in the literature. B. A shrunken apoptotic cell, from a meningioma cell line, showing segmented nucleus (N), lipid droplets (L), and lysosomes (Ly.). C. Apoptotic cell (circled, from a developing mouse eye). Notice the homogeneous appearance of the nucleus in apoptosis which differs from necroptosis. D. Apoptotic body in a developing mouse eye. Notice that the cellular contents are contained within the apoptotic body, and nothing leaked out. E. Apoptotic bodies inside a macrophage within a vessel from a developing mouse eye. F. Advanced stage apoptosis and the formation of the apoptotic body in a developing mouse eye. A remaining mitochondrion appears in a state of degeneration (inset).

Ultrastructurally, we recognize two modes of apoptosis: 1) developmental in embryonic and developing tissues, and 2) regulatory (homeostatic) occurs in the mature tissues and probably in their aging cells. The first mode does not appear to accumulate lipid droplets, autophagosomes, lysosomes, or vesicles. The second does; the states of their mitochondria

differ as well (compare Figs. 1A & B). Contrary to published reports, no cytoplasmic blebbing or break up of the cell was seen by EM; however, blebbing may take place in cells grown in tissue culture. Additionally, some apoptotic cells showed chromatin leakage (extranuclear DNA [enDNA]) in the cytoplasm (Fig. 3A), a feature that is absent from the apoptosis literature.

Inhibiting Apoptosis as A Clinical Option

Anti-apoptotic drugs. Minocycline use in acute ischemic stroke and other neurodegenerative diseases has been reported in many publications[17-24]. Minocycline, a 5-lipoxygenase inhibitor, is a systemic tetracycline that inhibits bacterial protein synthesis by binding to the 30S bacterial ribosome. It is used to treat skin or soft-tissue infections, mycobacterial infections, and nocardiosis [25]. Minocycline has shown activity in mediating neuroprotection by indirectly inhibiting the activity of caspase-1, caspase-3, the inducible form of nitric oxide synthetase (iNOS), and p38 mitogen-activated protein kinase (MAPK), which are known factors in amyotrophic lateral sclerosis (ALS); thus, theoretically delaying the progress of ALS in affected individuals. The extent of the benefit of Minocycline for ALS patients is still under investigation; however, some promising results have been published[26,27].

The anti-apoptotic effect of Minocycline, by inhibiting caspases and downregulation of cytochrome c release from the mitochondria, can act as a neuroprotective in acute stroke. Although treatment with tissue plasminogen activator (tPA) remains the mainstay for acute ischemic stroke, administering Minocycline after tPA has shown potential therapeutic benefits. This effect decreases the matrix metalloprotease (MMP) levels that increase after tPA treatment[28-30].

Ursodeoxycholic acid (UDCA) inhibits apoptosis through intrinsic or extrinsic pathways, reduces reactive oxygen species formation, and prevents mitochondrial dysfunction[31-35]. Furthermore, UDCA has shown benefits in lowering prion conversion and neuronal loss[36].

Due to its anti-apoptotic effect, cyclosporine has been suggested as an addition to the treatment of myocardial infarction. It reduces the release of cytochrome c from the mitochondria [37]. However, a recent clinical trial showed no difference between intervention and control groups[38]. Cyclosporine use as an anti-apoptotic medication is still being studied for various medical conditions[39].

2. Necroptosis

As seen by TEM (Fig. 4A & B), the ultrastructural changes of necroptosis include early plasma membrane permeabilization and disintegration, translucent degenerate cytosol, swollen degenerate mitochondria, and heterogeneous disintegration of chromatin. The latter is most likely because of the heterogeneity of the process and the lack of ample ATP to complete it. Cell ruptures due to membrane permeabilization and cytosolic contents are released into the extracellular space—see Fig. 4B[1]. Necroptosis is a faster process than apoptosis and does not require as much energy input as is required for apoptosis. Descriptions of necroptosis do not mention some of the events we observed by TEM, such as the appearance of lipid droplets, the cytoskeleton collapse, or the shedding of ribosomes. Because it generates cellular debris, necroptosis could be a source of localized inflammation.

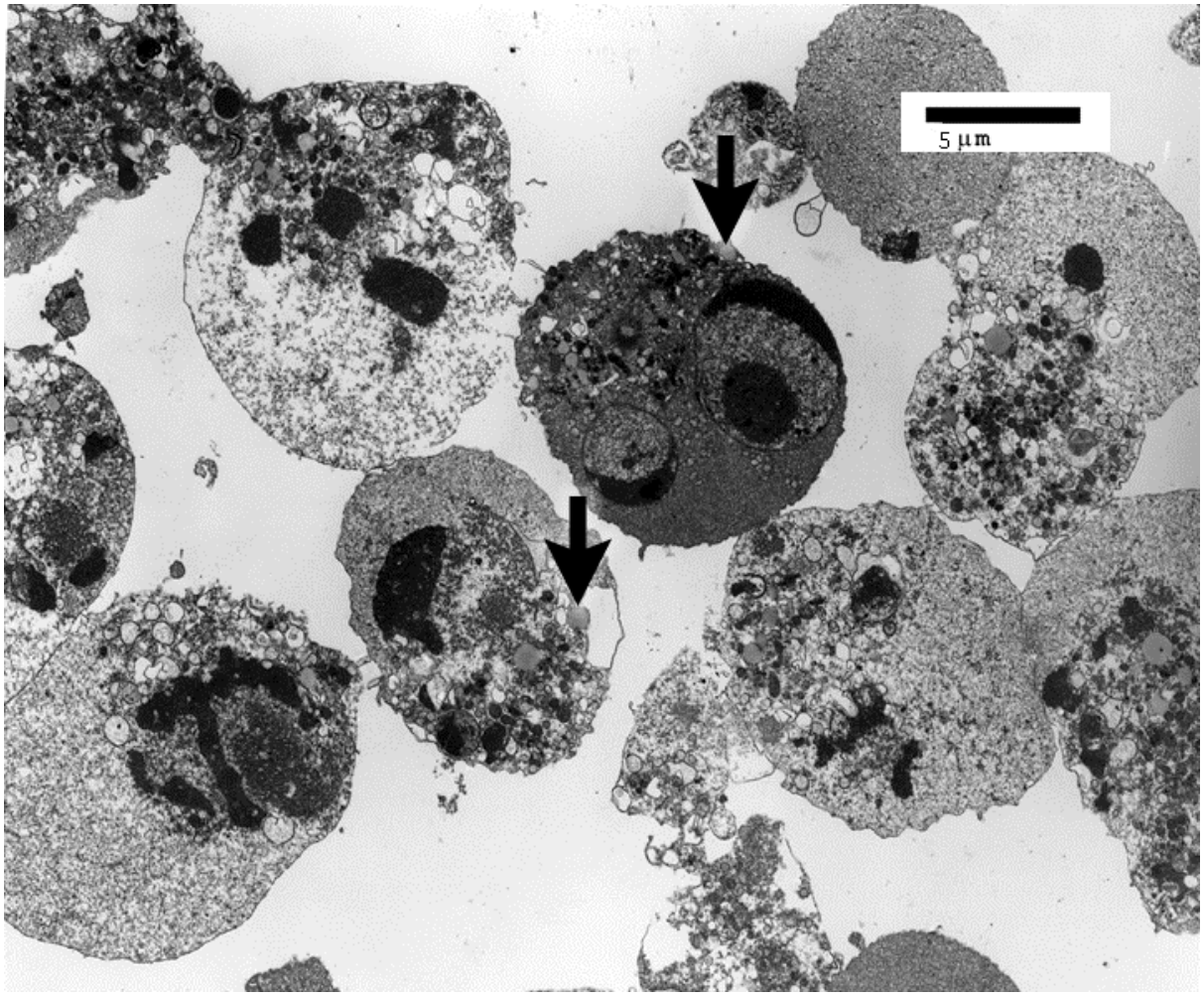

Figure 4. A.

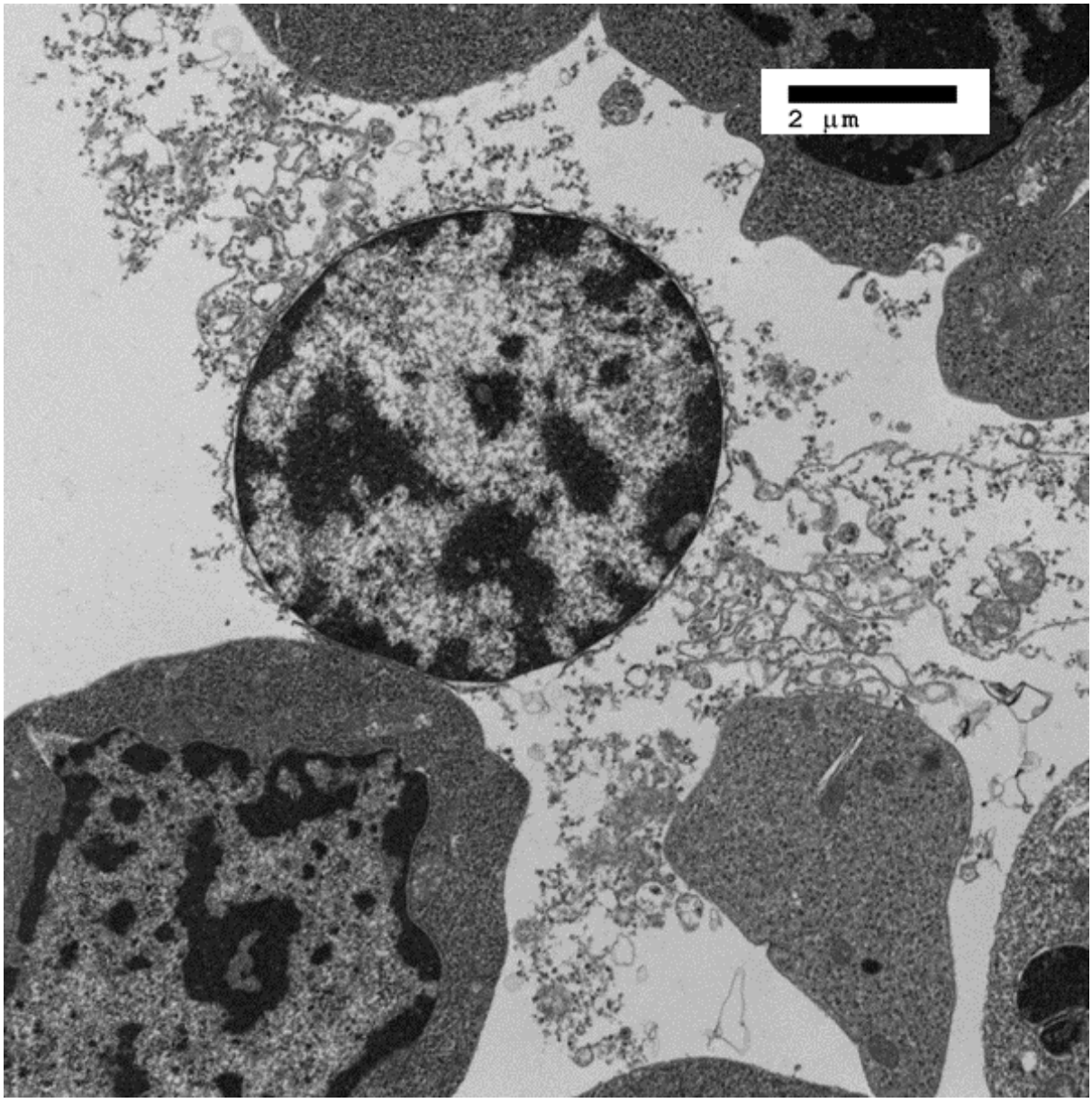

Figure 4. B.

Figure 4.
Necroptosis in bone marrow tissue. A. Cells at various stages of necroptosis. Notice plasma membrane permeabilization and disintegration, translucent degenerate cytosol, swollen degenerate mitochondria, and heterogeneous disintegration of chromatin. The cytosolic contents are released into the extracellular space. B. Necroptotic cell (center) without the degeneration of the nucleus.

The pathways for apoptosis and necroptosis are very different. Whereas the apoptotic pathways require the function of caspases, they work through RIPK3 in necroptosis[40]. Necroptosis can begin in a variety of paths. The most common is through damage-associated molecular patterns (DAMPs) using TLRs and TNFR1. Detection of short dsRNA

or dsDNA can also activate necroptosis. Several receptors trigger necroptosis: TNF, FAS, TRAIL, and TLR. These factors activate RIP1 and JNK, which lead to mitochondrial damage. DNA damage activates PARP, which depletes NAD^+ and subsequently ATP, thus causing necroptosis.

Kaczmarek et al. theorized that necroptosis could be a cellular defense mechanism against viral attack. The detection of viral machinery leads to a speedy suicide to prevent viral replication[41]. Indeed, viruses, including the *Vaccina virus*, encode caspase-8 inhibitors to avoid apoptosis. Mice infected with the *Vaccina virus* develop tissue necroptosis and neutrophil infiltration. Furthermore, deletion of RIPK3 lowers tissue inflammation but increases viral titers in their organs.

Under the same stimulus, young fibroblasts die by apoptosis while old (senescent) ones die by necroptosis[42]. Caspase inhibitors failed to prevent necroptosis of BCR-ABL-positive human leukemic cells treated with imatinib (Gleevec). Apoptosis-resistant cells die via necroptosis only when autophagy is suppressed-for example, by the activation of AKT[43]. We hypothesize that the suppression of autophagy prevents apoptosis and leads to necroptosis because, as we have noticed by TEM, suppression of autophagy turns the mitochondria into a large autophagosome by the destruction of the inner membrane prevents the early release of cytochrome c.

3. Pyroptosis:

Pyroptosis is a pro-inflammatory lytic programmed cell death that has been defined as gasdermin-mediated programmed death[44]. Research on the topic is ongoing, and our knowledge in this regard is still incomplete[45].

A set of morphological changes associated with pyroptosis has been reported: nuclear pyknosis, DNA fragmentation, poration of the cellular membrane, potassium leakage, intra and extracellular ion imbalance, cell swelling (once), and rupture and release of cellular contents[1]. However, published works on pyroptosis have not emphasized a mitochondrial role, accumulation of lipid droplets (Fig. 5A, B, E, F, & I) and glycogen (Fig. 5F), ribosomal shedding, or cytoskeletal changes (Fig. 5A-J). Furthermore, the cellular aberrations induced by IL-17A showed a different pattern of nuclear damage that had not been reported before where the chromatin condensation formed a ring around the inner side of the nuclear envelop and degenerate chromatin in the rest of the nucleus (Fig. 5 G & J). These observations were reported by our laboratory[46,47]. Unfortunately, they were not picked up by molecular-only driven research.

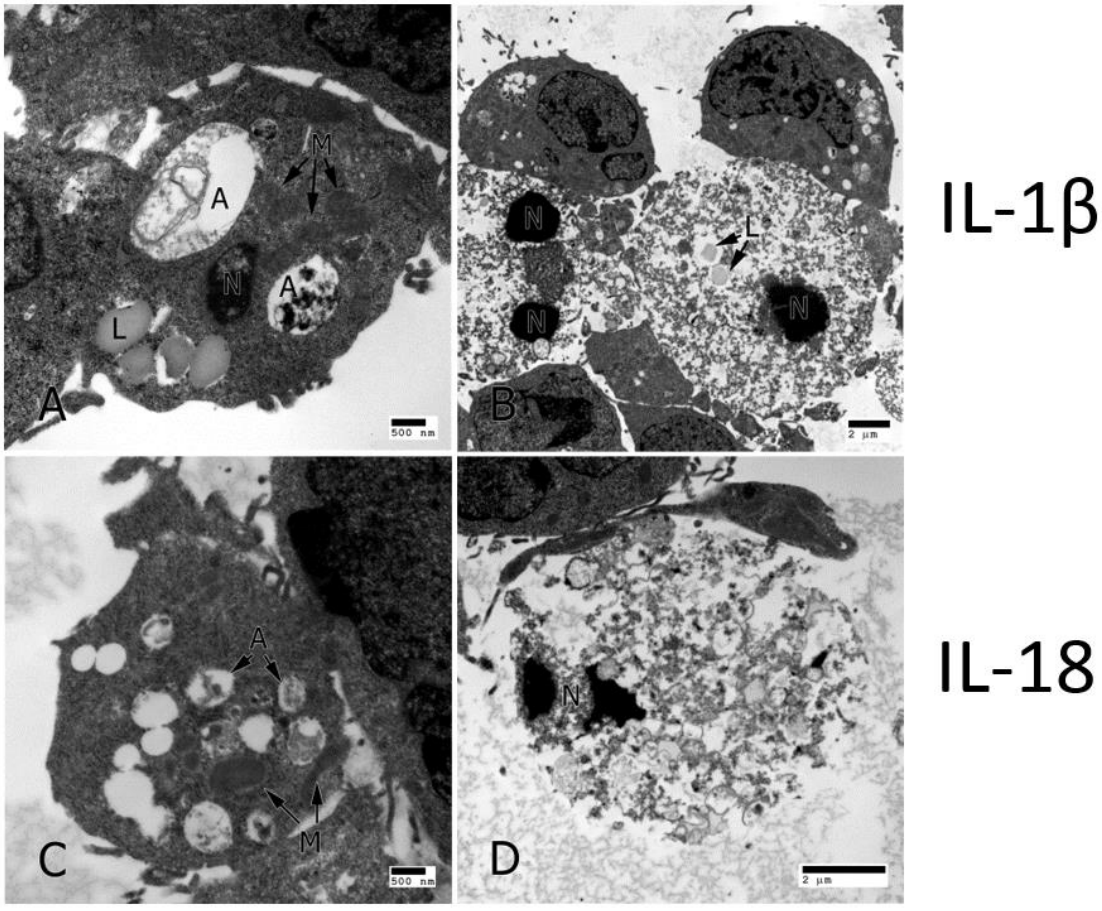

Figure 5 A-D

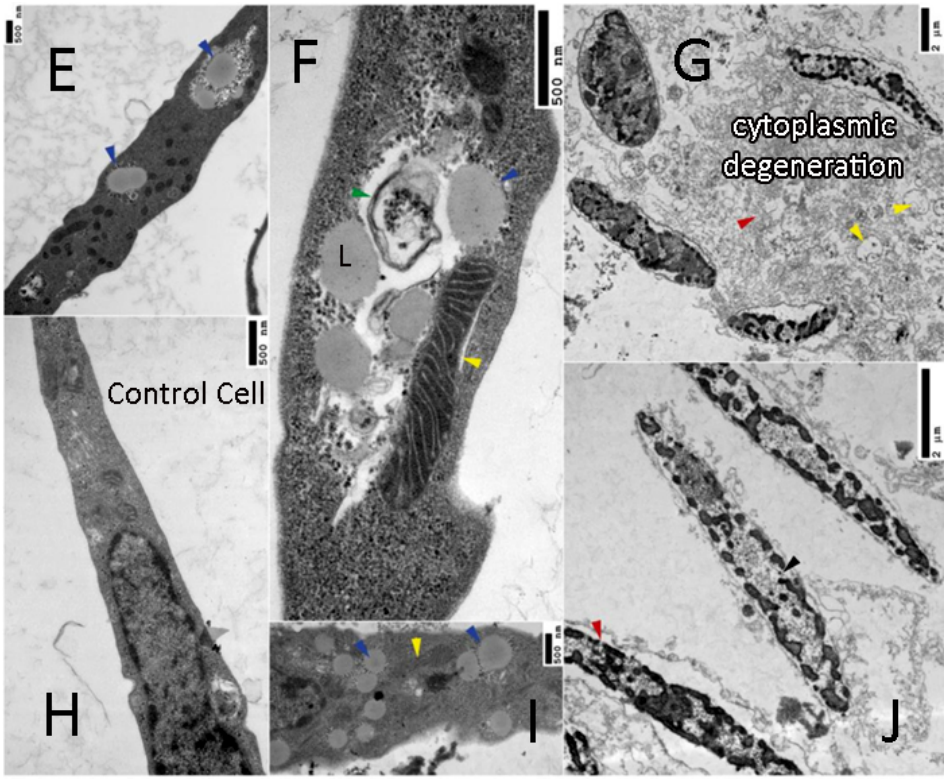

Figure 5 E-J

Figure 5

A-D. Pyroptosis in mouse retinal stem cells (RSC). A & B. IL-1β induced pyroptosis showing mitochondrial (M) damage, autophagic (A) vesicles, glycogen accumulation (not labeled), lipid droplets (L), and advanced disintegration of cytoplasm and nuclear condensation (N). C & D. IL-18 induced pyroptosis showing mitochondrial (M) damage, autophagy (A), and advanced cytoplasmic disintegration and nuclear condensation (N). Images from Ardeljan et al.[46].

E-J. IL-17A induced pyroptosis in ARPE-19 cells. Notice the accumulation of lipid droplets, glycogen, and mitochondrial damage. There is also cytoplasmic degeneration, as well as chromatin and nuclear membrane damage. Glycogen appears as black dots in F. Images from Ardeljan et. al.[47].

Second Class: Deaths Types Not Involving Death Signaling

1. Necrosis

Necrosis (from the Greek "death, the stage of dying, the act of killing") is a catastrophic, fast, and irreversible process triggered by external factors and causing extreme physicochemical stress on the cell. The stress could be induced by hypoxia, trauma, poisons, drugs, pathogens, radiation, or an autoimmune response elicited by the body itself[48]. As observed by EM, different stressors produce different morphological necrotic features. For example, hypoxia has the classic form of necrosis, where degeneration occurs simultaneously within all the organelles (Fig. 6). Coagulative (due to protein denaturation) is drug-induced (Fig. 7), while liquefactive (digestive) is of a pathogen infection such as a virus (Fig. 8).

Unlike apoptosis, necrosis does not require a death signal or energy to occur, and it is a pro-inflammatory process. Furthermore, necrosis may trigger poration of the nuclear envelope and causes the extrusion of chromatin from the nucleus to the cytoplasm (Fig. 9), an immunologic event that induces the release of INF-β through the cGAS-cGAMP signaling pathway[49].

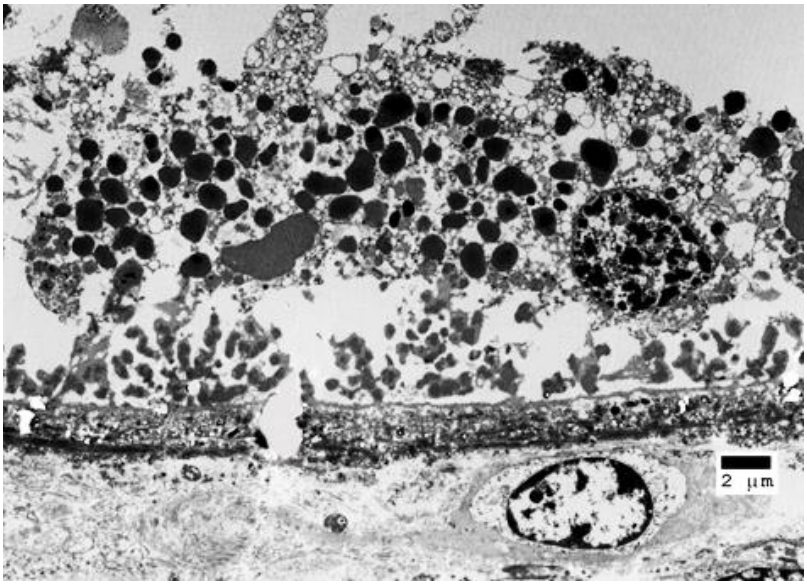

Figure 6.
Necrotic retinal pigment epithelial (RPE) cells from the eye of a Gaucher disease patient.

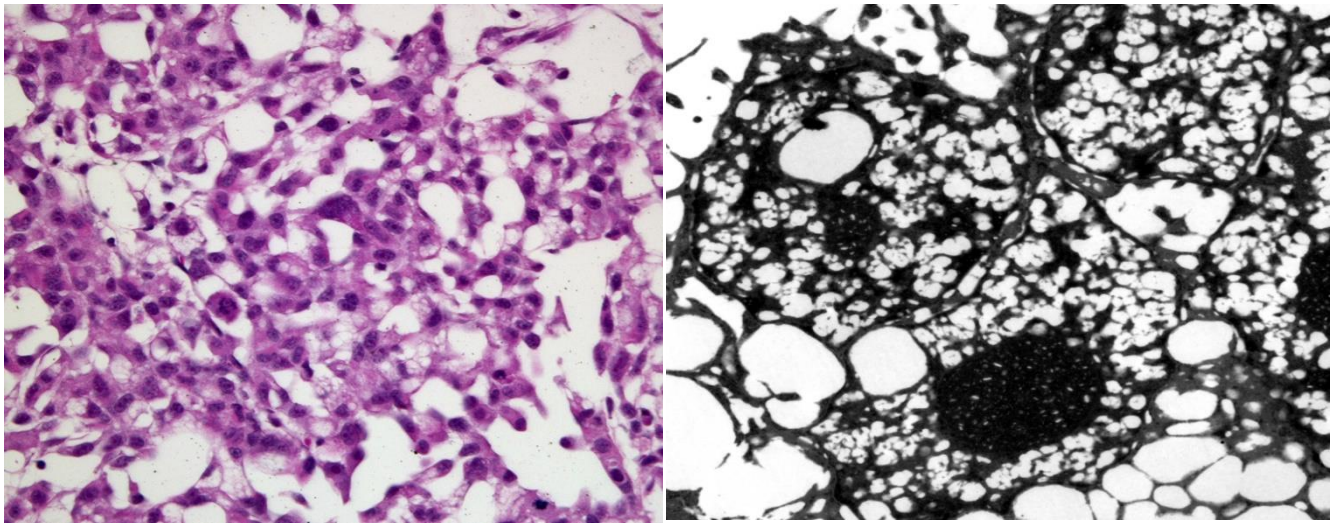

Figure 7.
Necrosis induced in prostate cancer tumor by treatment with an alcohol extract of saw palmetto.

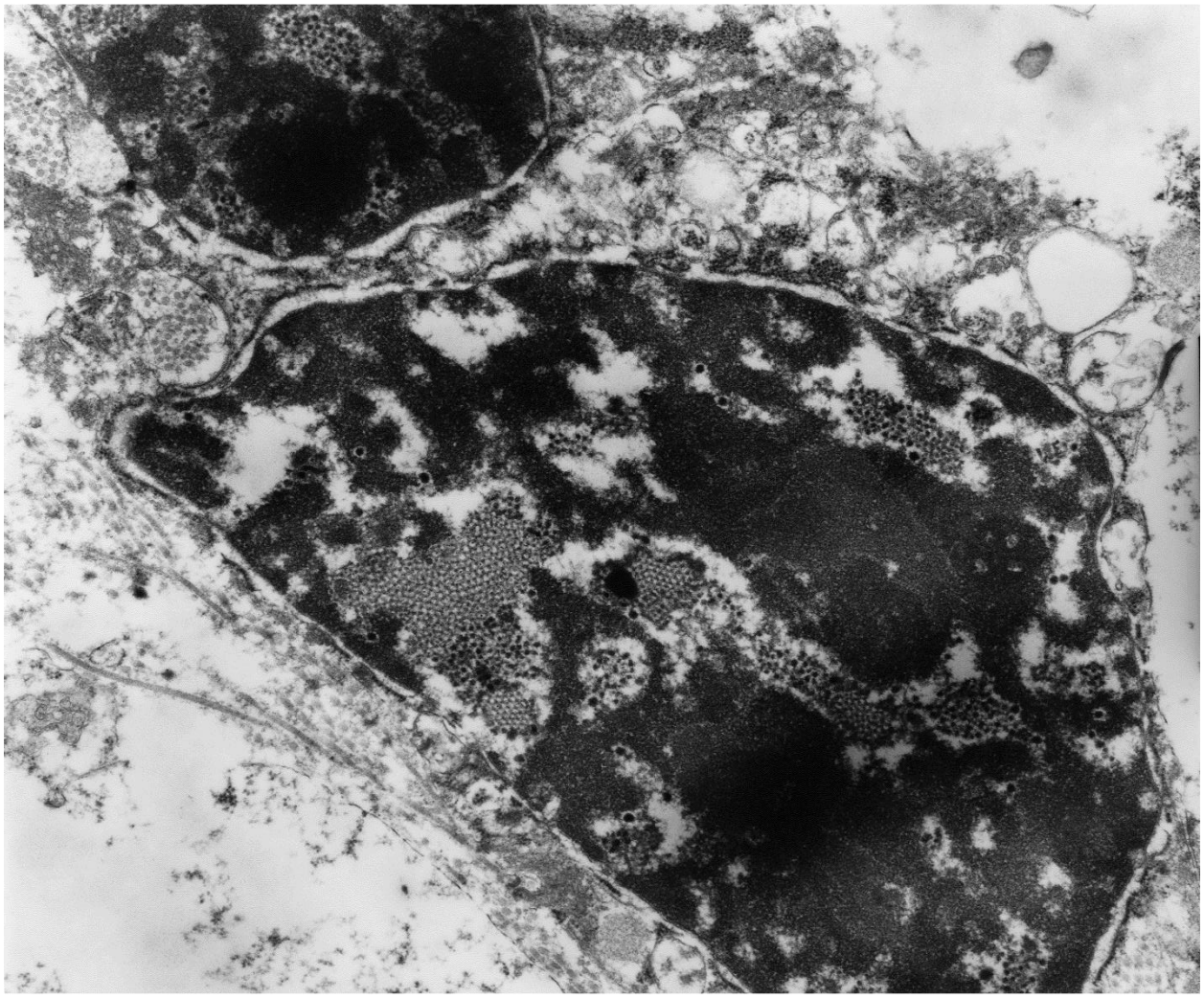

Figure 8.
The remains of a cell infected with the polyomavirus undergoing liquefactive necrosis. The major part remaining of the cell is the nucleus, which is filled with virus.

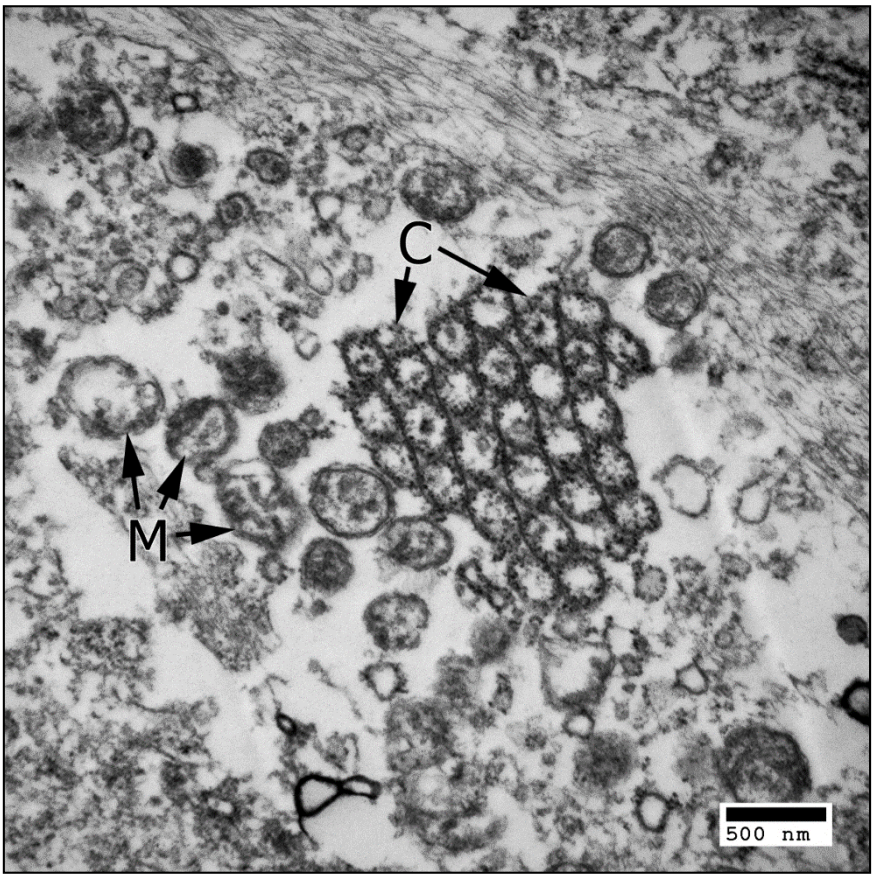

Figure 9.
Extranuclear DNA from an RPE cell of a patient with advanced AMD showing extranuclear chromatin (C) and mitochondrial disintegration (M). Chromatin strands form vesicles that clump together to form a lattice.

2. Mitophagoptosis: Autophagic Cell Death

Autophagosomes are membrane-bound vesicles that the cell generates in response to starvation, toxins, mitochondrial damage, and other causes[2]. Although the literature points to multiple intracellular sites, such as the endoplasmic reticulum (ER), Golgi apparatus, and the plasma membrane, as the origin of autophagosomes, the type that could lead to death is generated by the mitochondria in a process termed mitophagy[2].

In addition to hypoxia, starvation, and hyperthermia[6], mitophagy is activated by toxins and xenochemicals. Therefore, it plays a role in cellular survival by cleansing the cell of xenochemicals[50]. Mitophagy maintains mitochondrial homeostasis by excising damaged parts as mitophagosomes[51]. Most likely, mitophagy is an ancient evolutionary mechanism with roots in exocytosis. We have previously shown that autophagosomes originate from the mitochondria after drug treatment[51-53]. Autophagosomes may sequester xenochemicals, and rather than fusing with a lysosome, they connect with the cell membrane and dump their contents outside the cell, thus giving the cell a chance to survive. However, the continued exposure to the toxin causes excessive autophagosome formation and the destruction of the mitochondria, and the subsequent death of the cell[51].

We have observed that the continued

production of autophagosomes causes cell death by exhausting the mitochondrial population[51]. Excessive generation of autophagosomes leads to cell death, as seen in a mouse model of septicemia where the intestines were blocked (Fig. 10). The image shows a field of vacuoles that are autophagosomes produced by mitophagy in an

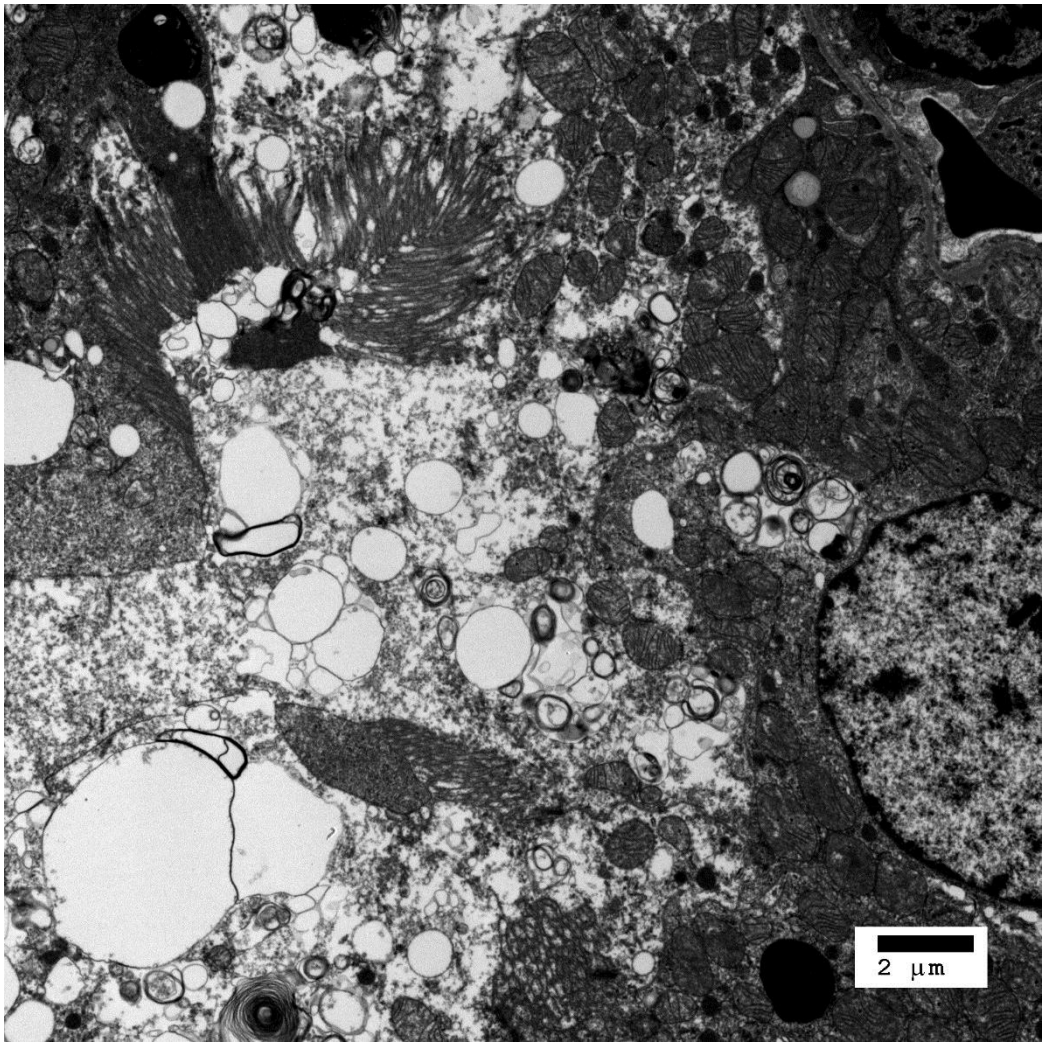

Figure 10.
Mitophagoptosis: Autophagic Cell Death. The TEM micrograph shows the epithelial cell of proximal tubule of mouse kidney after 18 hours of the beginning of septicemia model. All the vacuoles visible in the image are autophagosomes produced by mitophagy. Excessive production of autophagosomes by the mitochondria caused the degeneration of mitochondria and exhaustion and depletion of the mitochondrial population. Other intact mitochondria are swollen with visible disruption of the inner membrane.

epithelial cell of the proximal tubule of mouse kidney after 18 hours of the beginning of septicemia. Initially, autophagoptosis does not cause plasma membrane permeabilization; however, the membrane is damaged at later stages, and the autophagosomes can be seen in the urine by EM (unpublished data). As expected, mitophagoptosis is a disruptive and messy process that can cause an immune reaction.

Mitophagoptosis is not a programmed cell death. Mitophagy is a mitochondrial mechanism that evolved to protect the mitochondria from harmful foreign objects or cationic chemicals (basically, an exocytosis-like mechanism at the mitochondrial level). However, this mechanism goes on autopilot and does not stop as long as the toxin keeps entering the mitochondria, which means that it can only save the mitochondria (and the cell) if the assault stops before total depletion of the mitochondrial population. We have hypothesized that mitophagy is responsible for drug resistance in chemotherapy[51].

The literature on autophagy repeats the mantra that autophagosomes fuse with the lysosomes, forming autolysosomes[54]. However, based on our EM observations, we believe that this is not always the case and that autophagosomes themselves could become lysosomes, i.e., the lysosomes could have originated from mitochondrial autophagosomes.

Are There Other Types that Have Not Been Categorized?

This review is not aiming to be a comprehensive listing and characterization of cell death types since, without a doubt, some death processes have not yet been adequately studied. For example, we have encountered death types by toxins and drugs that do not fit the significant types outlined here. In a condition that we observed and termed Uniform Cellular Disintegration (UCD), all organelles showed degeneration with the same degree without cellular shrinkage or leakage of cellular and nuclear membranes and uniform degeneration of cytoplasm and the nuclear material. Furthermore, new modes of cell death such as entosis, methuosis, and paraptosis have been recognized, but we have not yet encountered them.

We also consider death by excessive chromatin leakage as a type of cell death, although it has not yet entered the literature as such. In certain age-related inflammatory conditions, the chromatin leaks outside the nucleus and into the cytoplasm, thus aggravating the inflammation and causing an escalating vicious cycle of inflammation that results in more damage to the chromatin and other cellular organs, leading eventually to cellular death. We have reported on such a condition in a mouse model of AMD and verified by immunohistochemistry that chromatin was in the cytoplasm[49].

Discussion

Despite the voluminous literature on cell death, there is still much more about cell death that has not yet been discovered. As we try to illustrate in this review, ultrastructural aspects and insights are not usually included in most published research. Therefore, there is an unmet need to couple the molecular data with the ultrastructural interpretation to reveal the panoramic details of the death. For example, we have shown that there is variation in apoptosis depending on the tissue where it is occurring, where apoptosis in developing tissue has a different morphology than homeostatic apoptosis.

Two ultrastructural features, the existence of lipid droplets and glycogen, are not reported in cell death, although they indicate cell distress. In several programmed death modes, such as apoptosis and pyroptosis, the sequence of their appearance (i.e., early v. late event) is unknown. For this reason, it is still unknown if the presence of lipid droplets signals impending cell death.

Thus far, we have observed lipid droplets in the cytoplasm of apoptotic, necroptotic, and pyroptotic cells (Figs. 3B, 4A, & 5A, F). The appearance of lipid droplets in the cell seems to be related to mitochondrial health and the dysfunctionality of the Tricarboxylic Acid Cycle (TCA). There is evidence that their accumulation following mitochondrial dysfunction is an evolutionarily conserved mechanism signifying an early transient indicator and promoter of neurodegenerative disease[9]. Furthermore, reducing lipid droplet accumulation in glia and lipid peroxidation by targeted lipase overexpression and/or lowering ROS (reactive oxygen species) delays the onset of neurodegeneration significantly[9].

Apoptosis is more pronounced in embryonic and developing tissues and nervous system cells where inflammation must be

avoided (e.g., outer and inner nuclear layers of the retina). Some tissues are not known to undergo apoptosis but rather die by necrosis, especially when perfusion becomes restricted. For example, the retinal pigment epithelium (RPE) cells exclusively undergo necrosis, thus causing age-related macular degeneration or AMD[55]. Additionally, apoptosis would not be possible in cancers, and restoring the defective apoptotic machinery in cancer (as a way to treat cancer) is far-fetched because the mitochondria are impaired in cancerous cells, and the cells display a hypoxic phenotype. Thus, they lack the energy to carry out and complete apoptosis[56].

Except in apoptosis, the mitochondrial role has not been well characterized ultrastructurally in other death mechanisms. In addition to mitophagoptosis, mitochondrial outer membrane permeabilization (MOMP) can initiate potent pro-inflammatory effects, including mitochondrial DNA-dependent activation of cGAS-STING signaling leading to a type I interferon response and subsequently causing non-apoptotic cell death[57]. Another non-apoptotic mitochondrial cell death is parthanatos, which involves PARP, PAR, and AIF and cannot be inhibited by caspase inhibitors[58]. Parthanatos has not been characterized ultrastructurally.

Chromatin leakage (or extranuclear DNA [enDNA]) is an under-reported phenomenon, and its association with pathology and cell death has not been well studied and characterized. We have shown by EM that chromatin leakage occurs in apoptotic cells (Fig. 3A), an association not reported in the literature. On the contrary, apoptosis is supposed to prevent chromatin leakage[59]. We discussed above that excessive chromatin leakage could cause cell death (not necessarily by apoptosis) because of its devastating inflammatory consequences[49]. However, it is still unknown whether apoptosis is an early or a later event in the death process.

Conclusion

There is an apparent disconnect between the molecular descriptions and the ultrastructural picture in cell death modes. This is especially pronounced when looking at the disintegration of cells, particularly in pathological conditions. Death, like all pathologies, is an energy-related process; thus, without mitochondrial dysfunction, there would not be death initiation. Death modes, as seen by EM, and variations on known ones need molecular elucidation, and at the same time, molecular descriptions of death modes are not adequate without ultrastructural characterization. A complete clarification of cellular death could be utilized in treating various diseases.

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موت الخلية من منظور البنية فائقة الصغر

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الملخص

في أبحاث مسارات موت الخلايا، لا تزال هناك فجوة واسعه بين النماذج التي تُقدمها الدراسات الجزيئية ودراسات الأنماط الشكلية التي يقدمها المجهر الالكتروني (TEM). ولأن عدداً قليلاً جداً من الأبحاث المنشورة تضمنت صوراً بالمجهر الإلكتروني، فإن العديد من ميزات البنية فائقة الصغر (ultrastructural) لم يتم دمجها بعد في أوصاف نماذج الموت. تشمل الميزات الشكلية التي تظهر في الخلايا المحتضرة، ولم يتم دمجها بعد في وصف نماذج الموت، تراكم قطيرات الدهون وتجمعات الجليكوجين، وظهور الكروماتين خارج النواة في السيتوبلازم، بالإضافة إلى المسارات المختلفة التي تؤدي الى خراب الميتوكوندريا. نحن نجادل أنه يجب استخدام المجهر الإلكتروني بشكل دائم في هذه الدراسات لأنه يُظهر بعض الميزات الجديدة التي لا تكشفها الدراسات الجزيئية، وأنه كان ناجحاً للغاية في التعرف على أنماط جديدة لموت الخلية مثل "إنتوسس" (entosis)، "مثيوسس" (methuosis) ، و"باراتوسس" (paraptosis). من الممكن أن يكون توضيح التسلسل الدقيق للأحداث في مسارات موت الخلايا هو حجر الزاوية لتقديم العلاج المناسب للعديد من الأمراض عن طريق إبطاء أو وقف تسلسل الانحطاط الخلوي. نقدم في هذه المراجعة تجربتنا الخاصة في تطبيق تفسيرات البنية فائقة الصغر لأنماط الموت وشرح أبعادها البيوكيميائية. من خلال القيام بذلك، نُكَمل البيانات الجزيئية والبيوكيميائية الحالية ونشير إلى الميزات المفقودة التي يجب أخذها في الاعتبار ودراستها في المستقبل.

الكلمات الدالة: موت الخلايا المبرمج (أبوتوسيس)، موت بالبلعمة الميتوكندرية (مايتوأتوبتوسيس)، موت بالإشعال المبرمج (بيروبتوسيس)، موت بالتنخر (نكروسيس)، موت بالتنخر المبرمج (نكروبتوسيس)، تسرب الكروماتين، جليكوجين، حامض نووي غير ريبوزي خارج النواة، قطيرات الدهون.