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TOPICAL REVIEW

Apoptosis: its origin, history, maintenance and the medical implications for cancer and aging

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Abstract

Programmed cell death is a basic cellular mechanism. Apoptotic-like programmed cell death (called apoptosis in animals) occurs in both unicellular and multicellular eukaryotes, and some apoptotic mechanisms are observed in bacteria. Endosymbiosis between mitochondria and eukaryotic cells took place early in the eukaryotic evolution, and some of the apoptotic-like mechanisms of mitochondria that were retained after this event now serve as parts of the eukaryotic apoptotic machinery. Apoptotic mechanisms have several functions in unicellular organisms: they include kin-selected altruistic suicide that controls population size, sharing common goods, and responding to viral infection. Apoptotic factors also have non-apoptotic functions. Apoptosis is involved in the cellular aging of eukaryotes, including humans. In addition, apoptosis is a key part of the innate tumor-suppression mechanism. Several anticancer drugs induce apoptosis, because apoptotic mechanisms are inactivated during oncogenesis. Because of the ancient history of apoptosis, I hypothesize that there is a deep relationship between mitochondrial metabolism, its role in aerobic versus anaerobic respiration, and the connection between apoptosis and cancer. Whereas normal cells rely primarily on oxidative mitochondrial respiration, most cancer cells use anaerobic metabolism. According to the Warburg hypothesis, the remodeling of the metabolism is one of the processes that leads to cancer. Recent studies indicate that anaerobic, non-mitochondrial respiration is particularly active in embryonic cells, stem cells, and aggressive stem-like cancer cells. Mitochondrial respiration is particularly active during the pathological aging of human cells in neurodegenerative diseases. According to the reversed Warburg hypothesis formulated by Demetrius, pathological aging is induced by mitochondrial respiration. Here, I advance the hypothesis that the stimulation of mitochondrial metabolism leads to pathological aging.

1. Background: basic definitions

Apoptosis is a type of PCD (programmed cell death) found in animals, first described in 1972 [1]. The classical studies were performed on *C. elegans*, a research model established by Sydney Brenner [2]. Adult worms have a predetermined number of cells: an adult hermaphrodite has 959 cells, and an adult male has 1031 [3, 4]. Sulston and Horvith described the developmental fate of every single cell of *C. elegans* (see figure 1) from the embryo to the adult, as a 'family tree,' and discovered dying cells: the generation of the 959 cells in the hermaphrodite was accomplished by the death of 131 cells. This proved that apoptosis was programmed, not accidental. Later, mutations affecting apoptosis were described [5].

The morphological and biochemical hallmarks of apoptosis allow one to easily distinguish it from other types of cell death. When apoptosis begins, there is a membrane permeability transition, characterized by the breakdown of the inner mitochondrial transmembrane potential [6]. The next stage is characterized by chromatin condensation and nuclear fragmentation [3]. Then the cell breaks into membrane-bound, ultrastructurally well-preserved fragments that are ingested by macrophages, which prevents the induction of inflammation [1].

Although apoptosis was long believed to occur only in animals, apoptosis-like cell death has also been described in multicellular and unicellular eukaryotes. In unicellular eukaryotes, it is not clear whether

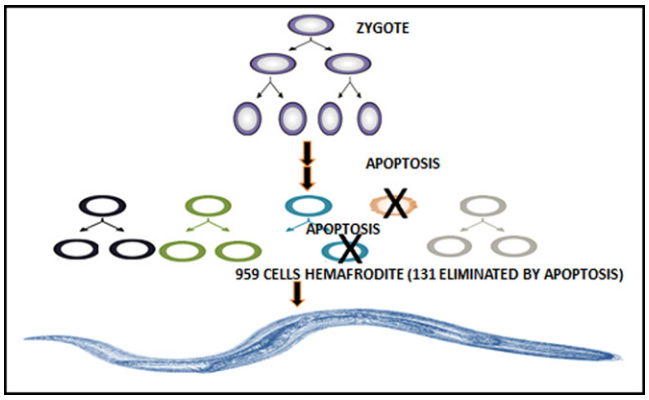

Figure 1. The map of developmental fate of the worm *C. elegans*. (The following image imported from wikipedia was used in this image: https://upload.wikimedia.org/wikipedia/commons/6/68/Turbellaria_003.png (public domain).)

apoptosis-like cell death is programmed or incidental [7]. There are also other types of programmed cell death in organisms that do not have mitochondria, including bacteria [8] and the amitochondrial protozoan *Trichomonas vaginalis* [9, 10].

2. Background: the main hypotheses on the origin of apoptosis

Two major hypotheses explaining the origin of apoptosis exist, and they are not mutually exclusive.

According to the first, the 'original sin' hypothesis, all living cells have an intrinsic inability to avoid random destruction. Cellular machinery regulates cellular mechanisms to repress suicide. This scenario provides a starting point for the evolution of programmed cell death: it views programmed cell death as a modification of accidental cell death [11]. Several recent papers support this hypothesis. For example, Proto *et al* [7] argue that the apoptotic-like cell death of parasitic protists may be incidental. Noticing that the proteins involved in programmed cell death also have other ancient functions, Dick and Megeney [12] suggested that the apoptotic behavior of this set of proteins was co-opted from core functions not originally associated with apoptosis.

According to the second hypothesis, the 'endosymbiotic' hypothesis, the apoptotic machinery originated in mitochondria. Kroemer observed that the release of apoptotic factors from mitochondria initiates apoptosis in the so-called permeability transition [6, 13]. Kroemer suggested that the interaction between the proto-mitochondrion and its initial host was analogous to the currently observed interaction between plasmids and bacteria [14]. Plasmids produce long-range toxins and short-range antidotes, allowing toxins to kill bacteria that lose plasmids [15]. His idea is supported by studies of the structural properties of the Bcl-2 proteins that both inhibit and activate animal apoptosis. Their structure is strikingly similar to the pore-forming domains of the bacterial colicin and diphtheria toxins [16], and, like bacterial toxins, they can form a channel through the cell membrane [17]. The BAX inhibitor 1 (BI-1) is a putative ancient anti-apoptotic factor (antitoxin). In a yeast model, Xu and Reed found that the transformation and expression of mammalian BAX induces yeast apoptosis and that although the yeast genome does not encode either BAX or BI-1, BI-1 is nevertheless a mammalian suppressor of yeast apoptosis induced by BAX [18]. The anti-apoptotic properties of BI-1 have since been demonstrated in both plants and animals [19]. BI-1 inhibits the release of apoptotic factors (toxins) from mitochondria rather than inhibiting their activity in the cytoplasm [20].

Frade and Michalidis [21] suggested that the proto-mitochondrion was a pathogen that induced the death of its host cell by a drop in levels of purines. According to this hypothesis, proto-mitochondrion was able to check the 'health' of the host against ATP levels. A high level of ATP would indicate that the cell is healthy. In contrast, decreasing amounts of ATP would be a signal indicating that the host cell is dying. In such conditions, the parasitic proto-mitochondrion would kill the cell and use its nutrients. Their hypothesis is based on the observation that the permeability transition results from the opening of mitochondrial transition pores, which are composed of adenine nucleotide translocators and mitochondrial porins. These proteins are related to eubacterial bacterial proteins.

The endosymbiotic hypothesis was later tested by Aravind *et al* with phylogenetic analysis [22]. They confirmed that many apoptotic factors encoded by the nucleus have a putative bacterial origin and suggested that these factors originated in mitochondria and moved to the nucleus through horizontal gene transfer. Their conclusion was that '*much of the glory of eukaryotic ascension to the ultimate complexity of higher plants and animals might be owed to a lucky choice of*

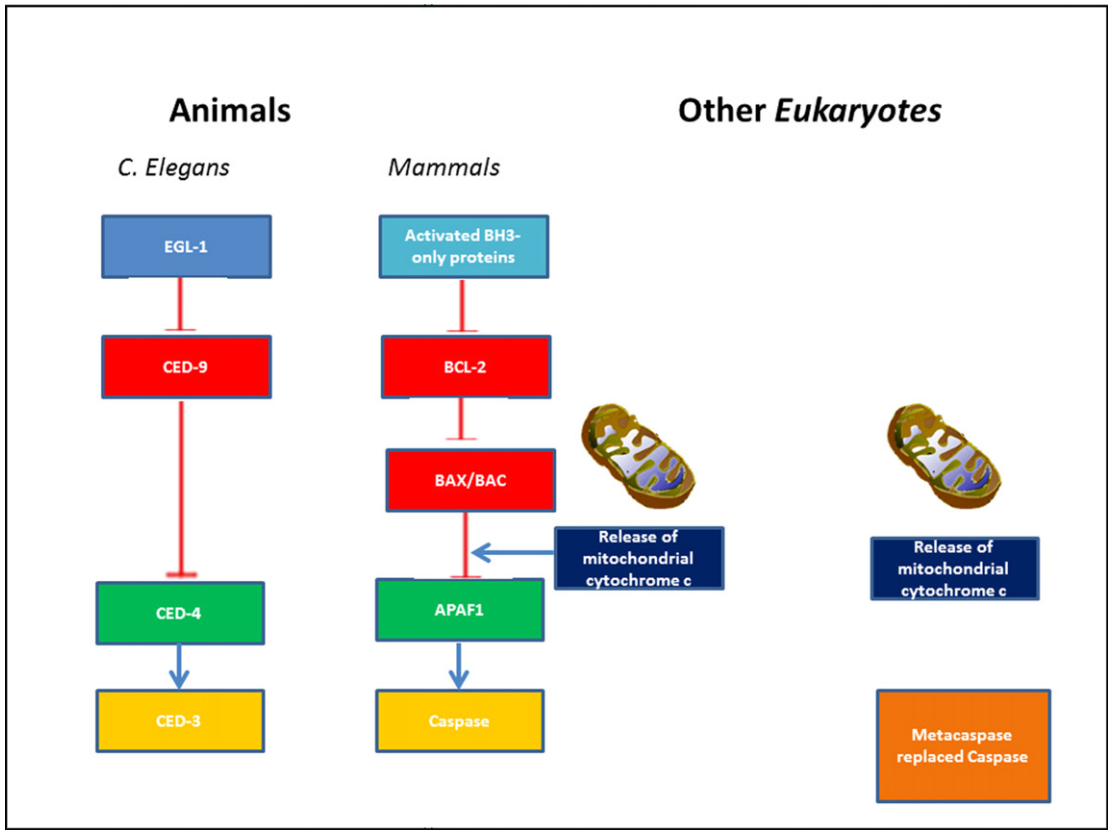

Figure 2. The evolution of 'canonical apoptotic pathway' from Degterev and Yuan [38]. The picture imported from wikipedia was used. (An image imported from wikipedia was used in this picture https://bg.wikipedia.org/wiki/%D0%9C%D0%B8%D1%82%D0%BE%D1%85%D0%BE%D0%BD%D0%B4%D1%80%D0%B8%D1%8F#/media/File:Mito.png (public domain).)

bacteria with complicated differentiation processes as the primary, pro-mitochondrial, and, perhaps, subsequent symbionts' [23].

The idea that apoptosis evolved due to a 'lucky choice' of bacteria with a complex regulatory system was further developed by Frank *et al* [24]. They suggested that bacterial sporulation resembles apoptotic mitochondrial fragmentation. However, in mammalian cell apoptosis, fragmentation of mitochondria is induced by dynamin-related protein 1 (Drp-1, Dynamin related protein 1). This factor is involved in mitochondrial division but is located largely in the cytosol. Under apoptotic conditions, cytosolic Drp-1 translocates into mitochondria. Inactivation of Drp-1 may directly prevent both apoptosis and apoptotic mitochondrial fission.

Frank *et al* also suggested that mitochondrial fragmentation derived from an early stress response leading to sporulation of the primitive mitochondria; it would have integrated stress signals and acted as an initial sensor for the eukaryotic response system.

3. Phylogenetic analyzes

Early studies revealed a linear apoptotic pathway whose core components are conserved from nematodes to humans, the 'canonical apoptotic pathway' [25–27] (see figure 2).

In *C. elegans*, cell death is induced by egg-laying defective (EGL-1) protein [28–30]. The activity of this gene modulates the effects of cell death abnormal (CED) mutations [28]. The EGL-1 protein interacts with the CED-9 anti-apoptotic protein [31]. The molecular mechanism of CED-9 anti-apoptotic activity is based on direct interaction with the CED-4 pro-apoptotic factor [32]. EGL-1 binding with CED-9 induces conformational changes in CED-9, resulting in dissociation of the CED-4 apoptotic activator from the complex [32]. The released CED-4 is then translocated to the perinuclear space [32], where eight CED-4 molecules form a funnel-shaped structure. This structure is the core of the apoptosome. The CED-3 caspase (apoptotic cystic aspartic protease) binds to this complex and establishes a holoenzyme. The activity of CED-3 caspase is markedly increased when complexed with apoptosome and induces cellular death [33].

In mammals, this pathway is more complicated, for the apoptosome also contains a mitochondrial cytochrome c [34–36].

Because the 'canonical apoptotic pathway' is not present in non-animal eukaryotes, some researchers incorrectly concluded that apoptosis occurs only in

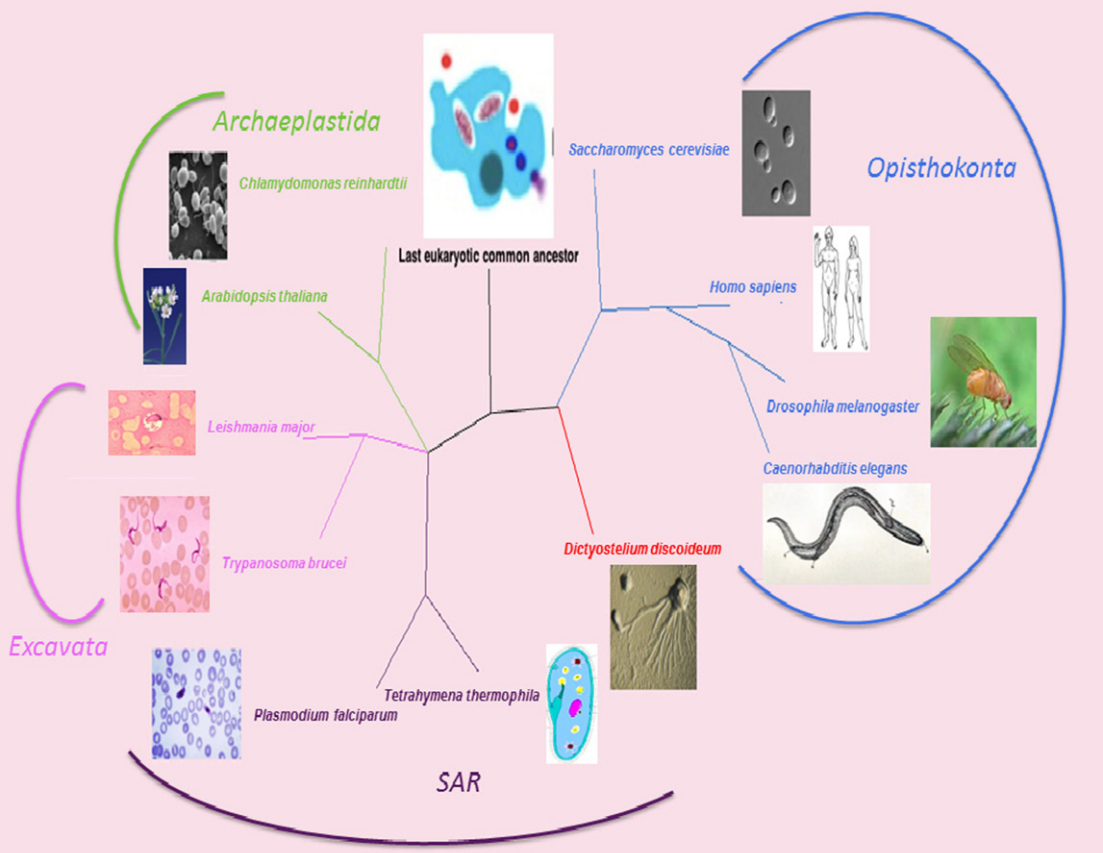

Figure 3. The phylogenetic tree used for inferences about the evolution of apoptosis. Only groups with well-described apoptosis-like cell death are included. All branches have 100% bootstrap support except the root position, which is controversial. From Kaczanowski *et al* [40], Adl *et al* [41], and He *et al* [39]. Pictures imported from wikipedia and Public Health Image Library of Centre for disease control and prevention were used in this image. (Following images imported from wikipedia were used in this figure: https://en.wikipedia.org/wiki/Chlamydomonas_reinhardtii#/media/File:Chlamydomonas6-1.jpg (public domain) https://commons.wikimedia.org/wiki/File:Arabidopsis_thaliana_flower.jpg (public domain) https://commons.wikimedia.org/wiki/File:Leishmania_donovani_01.png (Public Health Image Library of CDC-public domain) https://upload.wikimedia.org/wikipedia/commons/2/23/Trypanosoma_sp._PHIL_613_lores.jpg (Public Health Image Library of CDC-public domain) https://pl.wikipedia.org/wiki/Zarodziec_sierpowy#/media/File:Plasmodium_falciparum_01.png (Public Health Image Library of CDC-public domain) https://upload.wikimedia.org/wikipedia/commons/thumb/f/f9/Ciliophora_morphology.svg/512px-Ciliophora_morphology.svg.png (public domain) https://en.wikipedia.org/wiki/Dictyostelium_discoideum#/media/File:Dictyostelium_Aggregation.JPG (public domain) https://upload.wikimedia.org/wikipedia/commons/6/68/Turbellaria_003.png (public domain) https://commons.wikimedia.org/wiki/File:Drosophila-melanogaster-Nauener-Stadtwald-03-VII-2007-12.jpg (public domain) https://commons.wikimedia.org/wiki/Homo_sapiens#/media/File:Human.svg (public domain) https://commons.wikimedia.org/wiki/Saccharomyces_cerevisiae?uselang=pl#/media/File:S_cerevisiae_under_DIC_microscopy.jpg (public domain).)

animals. Later, more apoptotic mechanisms were discovered, some of which occurred in non-animal eukaryotes or bacteria. Programmed cell death induced by mitochondria is now believed to occur in a broad range of eukaryotic organisms [37].

I analyzed the evolution of programmed cell death in unicellular protists and multicellular eukaryotic organisms (figure 3) using a phylogenetic tree based on a recently published analysis of the evolution of 37 proteins [39]. (I assumed that barley [*Hordeum vulgare*] has the phylogenetic position of *A. thaliana*.)

Three mechanisms were conserved across lineages (see figure 4): (1) release of mitochondrial apoptotic factors (red); (2) induction of PCD by apoptotic proteases (caspases and metacaspases); and (3) destruction of nuclear DNA by apoptotic DNases (marked green).

We propose the following model for their evolution [40]:

Step 1: Release of mitochondrial apoptotic factors

The first conserved mechanism in our model is the release of mitochondrial apoptotic factors, including cytochrome c, apoptosis-inducing factor (AIF), and EndoG, as well as the nucleases and proteases involved in apoptosis. Cytochrome c is part of the mitochondrial oxidative chain [42]. The apoptotic activity of cytochrome c has been shown in both yeast [43] and animals [36], where it interacts with and enhances the activity of apoptotic proteases, caspases (animal apoptotic cystic aspartic proteases), or metacaspases (non-animal apoptotic lysine/arginine specific proteases) [43, 44]. Significantly, in animal cells, cytochrome c is also part of a multiprotein apoptosis-activating complex called the apoptosome [34–36]. The AIF is a

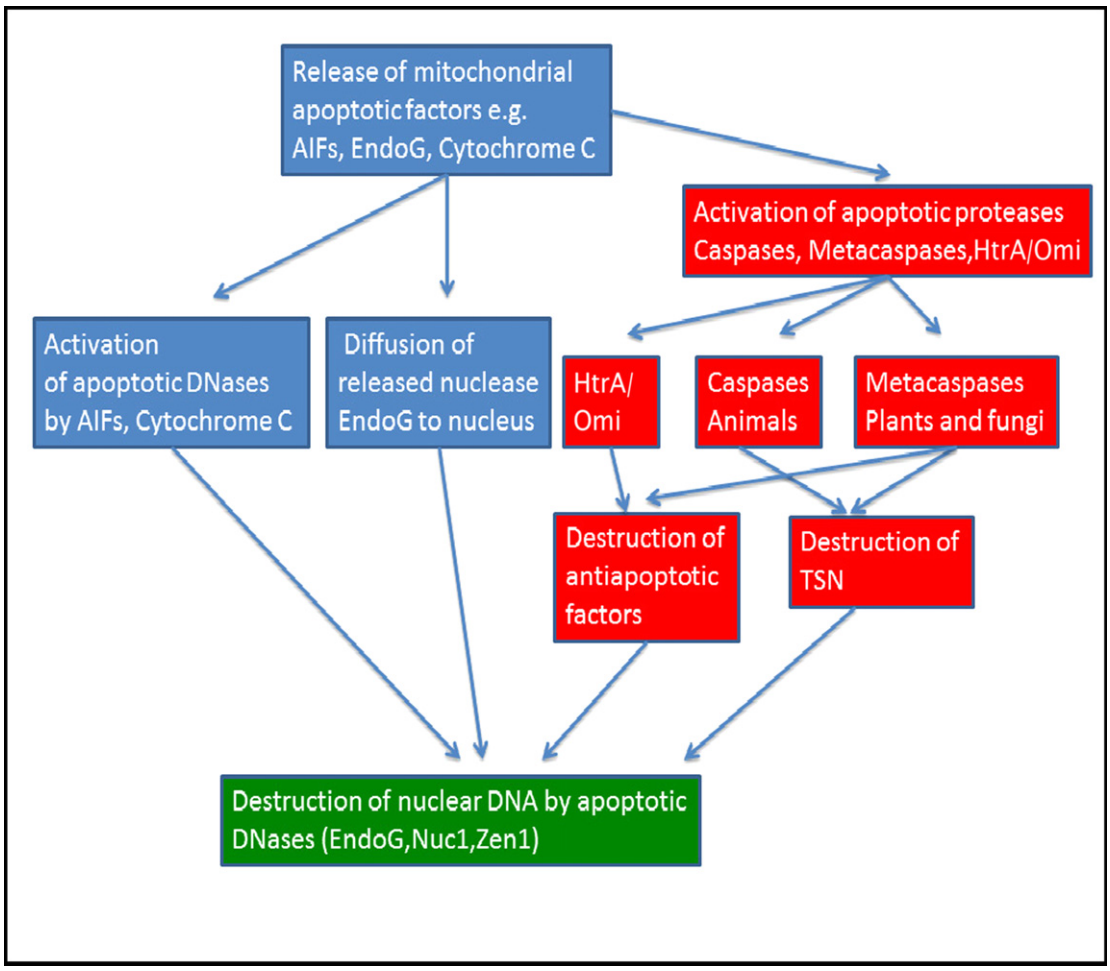

Figure 4. The scheme of evolutionary-conserved apoptotic pathways.

flavoprotein. Several studies indicate that it performs an important function in respiration and suggest a role for it in respiratory chain homeostasis [45]. The apoptotic activity of AIF has been demonstrated in organisms belonging to widely differing taxonomic groups: humans [46–48], yeast [49], slime molds [50], and *Tetrahymena* [51].

Interestingly, studies of human AIFs revealed that the flavoprotein domain is required only in mitochondrial AIFM1 [47] but is not required for the induction of apoptosis in AIFM2 [48] and AIFM3 [52]. This observation suggests that different parts of proteins function in apoptosis and respiration.

A transition in mitochondrial permeability plays an important role in the direct activation of some (but not all) apoptotic proteases and DNases. Caspase 9, for example, is released from mitochondria during apoptosis [53]. In contrast, caspase 2 is localized to the nucleus and the Golgi apparatus [54]. The animal apoptotic protease HtrA2/Omi [55] is also released from mitochondria during the apoptotic mitochondrial transition, but the yeast HtrA2 ortholog Nma111p exhibits nuclear localization [56]. Apoptotic DNA-ses EndoG [57] and TNM1 of *Tetrahymena* are released during apoptosis [58], whereas proteins belonging to the NUC1/apoptotic DNase 2 family have lysosomal localization [59, 60].

Step 2: Induction of apoptotic proteases

The second conserved apoptotic mechanism in our model is the induction of apoptotic proteases, the caspases, and metacaspases. Caspases are found in animals, whereas metacaspases are found only in eukaryotes that lack caspases: plants, protists, and fungi. Like caspases, they contain a caspase-specific catalytic diad of histidine and cysteine, as well as a caspase-like secondary structure.

Metacaspases and caspases have similar functions. The apoptotic function of metacaspases has been demonstrated in distantly related plants [61] and fungi [62], and that of caspases has been demonstrated in animals [53]. Caspases and metacaspases are so remotely related that they have different enzymatic specificity. Both types of protease have many different substrates. Among them is tudor staphyllococus nuclease, whose inactivation induces apoptosis, a process that is probably an ancient conserved apoptotic mechanism [63].

Both metacaspases and caspases also have non-apoptotic functions. For example, metacaspases play a role in cell cycle regulation in yeast and *Trypanosomes*

Table 1. BLAST homology searches using apoptosis DNases as queries. Results were confirmed using reciprocal blast. Each query DNase represents a different column and homologs detected in other organisms are presented in each row. The IDs for query and detected sequences are given and the expected values presented below each ID.

	CAD *D. melanogaster* **NP_609631.1**	ENDOG *H. sapiens* **NP_004426.2**	ZEN1 *H. vulgare* **BAA28942.1**	NUC1 *C. elegans* **NP_509604.1**	TNM1 *T. thermophila* **XP_001013802.2**
H. sapiens	**NP_004393.1** 7×10^{-25}	**NP_004426.2** **0**	**NO**	**NP_067056.2** 10^{-53}	**NO**
D. melanogaster	**NP_609631.10**	**NP_610737.1** 5×10^{-90}	**NO**	**NP_650672.1** 10^{-56}	**NO**
C. elegans	**NO**	**NP_491371.1** 8×10^{-77}	**NO**	**NP_509604.1** **0**	**NO**
S. cerevisiae	**NO**	**NP_012327.1** 2×10^{-56}	**NO**	**NO**	**NO**
D. discoideum	**NO**	**XP_637185.1** 9×10^{-28}	**NO**	**XP_635662.1** 2×10^{-53}	**NO**
V. carteri	**NO**	**XP_002957302.1** 10^{-38}	**NO**	**XP_002952503.1** **0.012**	**NO**
A. thaliana	**NO**	**NO**	**NP_680734.1** 10^{-100}	**NO**	**NO**
P. falciparum	**NO**	**NO**	**XP_002808920.1** 4×10^{-10}	**NO**	**NO**
T. cruzi	**NO**	**XP_813011.1** 5×10^{-25}	**XP_811600.1** 2×10^{-12}	**NO**	**NO**
T. thermophila	**NO**	**NO**	**XP_001012761.1** 6×10^{-12}	**XP_976867.1** 2×10^{-40}	**XP_001013802.2** 8×10^{-99}
T. vaginalis	**NO**	**NO**	**XP_001323878.1+** 3×10^{-9}	**XP_001312304.1** 2×10^{-48}	**NO**

[64]. A recent study shows that *Drosophila* caspase is required for cellular migration [65].

Step 3: Self-destruction of DNA

The third conserved mechanism in our model is the self-destruction of DNA by the cell's own nucleolytic DNases, including EndoG [57, 66–68], ZEN1 [69], NUC1/DNase II [59], CAD, and TNM1 [40] (see table 1).

We showed that EndoG, ZEN1, and NUC1 are ancient apoptotic factors shared among distantly related lineages [40]. The apoptotic function of EndoG has been demonstrated in various animals [57, 70], yeasts [67], trypanosomes [68], and *Leishmania* [66].

NUC1, ZEN1, and EndoG are ancient apoptotic DNases with homologs in remotely related eukaryotic taxonomic groups and bacteria. ZEN1 is a key apoptosis DNase in plants. We also observed apoptotic nucleases in one lineage. One of them, a well-described apoptotic nuclease, is the mammalian caspase-activated DNase, or CAD [71]. CAD is found in a complex with its inhibitor protein, ICAD, also called DNA fragmentation factor 45 [72]. It has been shown that cleavage of this inhibitor by caspases activates apoptotic degradation. These proteins are encoded by the genomes of mammals and insects [40]. It is likely that this enzyme appeared after the divergence of animals.

Another apoptotic nuclease observed exclusively in one lineage is the mitochondrial nuclease TMN1 of ciliates. Basic local alignment search tool (BLAST) searches indicate that this protein is encoded only by ciliates [58].

In conclusion, apoptosis is an ancient evolutionary mechanism with similar pathways in widely differing systematic groups. However, in each of the groups, elements of the pathways have been adapted to other functions as well.

Several bacterial program cell death mechanisms are described. They are often based on a toxin-antitoxin system [15]. It is not clear if any of such mechanisms are evolutionary related to apoptosis [73–75].

4. Debate about the maintenance of apoptosis

Apoptotic mechanisms are complex even in unicellular organisms. For example, in yeast, apoptosis-like cell death is induced by different stimuli, including aging [76], chemical or physical stress [77], and unsuccessful mating [78] (for a review, see [79]).

This observation prompts two great questions for evolutionary biology: what is the origin of apoptosis, and why is apoptotic machinery maintained in the extant organisms? In previous chapters, I discussed different hypotheses on the origin of apoptotic mechanism. However, it is not clear if such hypotheses provide an explanation for the maintenance of apoptosis.

There is a fundamental difference between the origin and maintenance. The origin of apoptotic traits was caused by selection forces acting in the past. The maintenance of such traits is caused by currently existing selection forces.

While it is not clear why complex apoptotic mechanisms are maintained in unicellular organisms, some scientists hypothesize that apoptosis is altruistic suicide maintained by kin selection [40, 80, 81]. According to this hypothesis, PCD is adaptive. However, 'altruistic suicide' is not an exclusive explanation for the maintenance of apoptosis. 'Antagonistic pleiotropy' is a second hypothesis also considered by different scholars. This hypothesis was first formulated by Williams as an evolutionary explanation of senescence [82]. According to this theory, antagonistic pleiotropy occurs when one gene controls many traits, some of them beneficial and others deleterious. This hypothesis could also be applied to apoptosis, as has been pointed out in a review by Garrido and Kroemer [83].

Different experiments indicate that there are circumstances in which the 'altruistic suicide' seems be the more favorable explanation, when in others, the negative pleiotropy is more likely.

There are several non-exclusive explanations why 'altruistic' suicide is beneficial for surviving cells.

The first is the involvement of programmed cell death in the regulation of cell density.

Several studies of protozoan parasites support this view. For example, *Trypanosoma,* which causes sleeping sickness, uses several mechanisms for cell density regulation [80]. One of them occurs in the developmental stage called 'stumpy forms.' Stumpy cells produce an inducer of cell death of stumpy forms—the prostaglandin D_2 (PGD_2). Apoptosis-like cell death has also been observed in malaria parasites—*Plasmodium*—during the infection of mosquitos [84]. The involvement of apoptosis-like cell death in the regulation of population size has also been described for free-living unicellular eukaryotes that cause phytoplankton blooms (for a review, see [85]). For example, a bloom of the dinoflagellate *Peridinium gatunense* is observed each year in Lake Kinneret. The apoptosis-like cell death quickly terminates the bloom [86].

The 'public goods' hypothesis is another plausible explanation of 'altruistic suicide.' According to this theory, the contents released by a cell programmed to die constitute a 'public good' that benefits surviving neighboring and related cells. In the green algae, *Chlamydomonas*, Durand's group [87] has shown that the contents liberated during non-programmed cell death are detrimental to other cells, whereas the contents released during apoptotic-like programmed cell death are beneficial. This observation suggests a possible mechanism for the origin and maintenance of apoptotic-like programmed cell death: it was initially a mechanism to eliminate toxic substances released by a dying cell and protecting related neighbors.

Later they showed that this beneficial effect of the liberated contents during program cell-death is species-specific. They showed that PCD has an inhibitory effect on the growth of other species [88].

Bacteria often respond to the attack of an obligatory lytic phage by committing suicide. This is an altruistic act protecting other cells. It has been shown, using synthetic biology, that the reproductive cost of suicide is low, because an infected cell has no chance of escaping death, and the act provides large benefits to related survivors [89]. It has also been shown that eukaryotic apoptosis-like programmed cell death is also involved in the analogous response to the attack of viruses in unicellular green algae *Emiliania huxlei* [90].

As was already mentioned, the negative pleiotropy hypothesis could also be applied to apoptosis, as has been pointed out in a review by Garrido and Kroemer showing that yeast apoptotic proteins also have non-apoptotic vital functions [83].

Different studies support this claim by different genetic studies: e.g., cytochrome c, apart from being part of the respiratory chain, is an apoptotic factor [91]. It has also been shown that metacaspase is involved in the process of clearing insoluble protein aggregates [92], and that inactivation of its gene results in slower cell cycle progression [93].

5. Evolution of developmental apoptosis

Multicellularity appeared several times independently during the eukaryotic evolution in plants, fungi, and animals. A recent paper has shown, using experimental evolution, that multicellularity can be rapidly selected in yeasts [94]. Gravity was the selection factor. Selected multicellular strains had higher rates of apoptosis-like cell death. In multicellular organisms, cell death is an important developmental mechanism called developmental apoptosis. Even in unicellular ciliates like *Tetrahymena*, primitive developmental cell death is observed. *Tetrahymena* have a complex life cycle. After sexual conjugation, one nucleus (called a macronucleus) is degraded by apoptotic-like cell death [95]. There are several other examples that show that developmental apoptosis is not exclusively an animal process, which is required to establish multicellularity. For example, it is described in the slime mold, *Dictyostelium* [96], as well as green plants (see [97]), where it is involved in root development [98] and the development of pollen [99]. In cnidarians, the most basal animal clade, apoptosis is crucial for metamorphosis leading to the establishment of germ layers [100, 101]. Apoptosis is also involved in the development of the nervous and immune systems in animals, including humans (see [102] and [103]). The critical function of apoptosis is not restricted to development. It also plays an important role in maintaining homeostasis in adult organisms. It is a crucial component of wound healing, which involves three phases: inflammation, tissue formation, and tissue remodeling. During this process, specific populations of cell types rapidly multiply or migrate to wounds. Later, such cells are removed by apoptosis (see [104]).

6. Evolution of apoptosis and cellular aging

Aging is an evolutionary inevitability observed in virtually all organisms. It is likely that this process appeared before the endosymbiosis of the proto-mitochondrion with the proto-eukaryotic cell and thus well before multicellularity. It appears that all organisms that divide asymmetrically must age. For example, the division of *E. coli* is asymmetric: the 'parent' cell inherits the older parts of the cell (e.g., the end or pole) and the 'daughter' cell is rejuvenated by the inheritance of newly synthetized parts [105]. This is called replicative aging, in which cells inheriting older components grow more slowly, divide less frequently, and have higher mortality rates. It is likely that the aging of *E. coli* is caused by protein aggregates, which are inherited asymmetrically [106].

Several examples suggest that in eukaryotes aging induces apoptosis. Replicative aging also occurs in yeast, a unicellular eukaryote, where accumulation of protein aggregates is observed in aging cells. The mechanisms of the cell death of aged cells in yeast and in bacteria differ slightly; old yeast cells often experience apoptosis-like cell death [107–109] (see [110]) in yeast, where deletion of pro-apoptotic factors increases life span [62, 111]. Apoptosis and the pathological accumulation of peptide aggregates play important roles in animal cellular aging, including humans. Several aged linked pathologies are caused by the accumulation of toxic protein aggregates [112] that can induce pathological apoptosis. Apoptosis seems be a very general aging mechanism. A classical study showed that the accumulation of mitochondrial mutations in mutant mice of mitochondrial DNA polymerase γ (POLG) induces apoptosis in different tissues [113]. It would appear that this induction of apoptosis accelerates animal aging [113].

6.1. Carcinogenesis and apoptosis: the Warburg hypothesis

I hypothesize that there is a deep relationship between mitochondrial metabolism, its role in aerobic versus anaerobic respiration, and apoptosis. Such a relationship can be observed in human cells during tumorigenesis, pathological aging, and neurodegenerative diseases

In contrast to aging cells, cancer cells are immortal. Different studies suggest that carcinogenesis reverses cellular aging. Understanding the origin of apoptosis helps us to understand why apoptosis has been deeply conserved and may shed light on the causes of cancer. A recent review suggested that a malignant transformation can be seen as a reversion from the phenotype of a differentiated cell of a multicellular organism to an ancestral unicellular eukaryotic phenotype [114]. Such rapid reversal of evolution may be one key to understanding cancer.

Mutations in mitochondrial DNA (mtDNA) tend to accumulate in cancer cells [115, 116]. The function of these mutations has been analyzed using cibrids (cytoplasmic hybrids) to place mitochondria into different cell lines; the results suggest that mtDNA has strong effects on the metastatic phenotype of cancer cells [117, 118].

During neoplastic transformation, the suppression of apoptosis is an obligatory compensatory change following the dysregulation of cell proliferation [119, 120]. Antiapoptotic inhibitors such as B-cell lymphoma 2 (BCL2) are overexpressed [121], and pro-apoptotic proteins, like those of the subunit of apoptosome apoptotic protease activating factor 1 (APAF1-1) and BAX protein (Bcl-2 associated X protein), are often inhibited in malignant cancer cells [35, 122].

Apoptosis is a key part of the intrinsic tumor suppression mechanisms (for a review, see [123]). Several stress types accompanying malignant transformation activate apoptosis. In animals, p53 is a major stress sensor involved in regulating the progress of the cell cycle as well as apoptosis (see figure 5). Mitochondria are usually a key player in apoptosis (although mitochondria- independent apoptosis also exists [124]).

Malignant transformation causes several perturbations of cellular activity (malignant stresses), among them defects in chromosome segregation. Such defects were first observed by Boveri in 1914 [125], who suggested that cancer arises from genomic instability, an assumption that remains prevalent in current thinking on cancer. Later, it was shown that elevated mutagenesis increases the probability of somatic mutation and malignant transformation (for a review, see [126]). Both genomic instability [127] and hypoxia [128] are often experienced by locally advanced tumors, creating stresses that activate P53-dependent apoptosis (for a review, see [127, 129]). There are also P53-independent apoptosis activation pathways. Hypoxia, for instance, also induces P53-independent apoptosis by activating genes from the BCL-2 family [128], and P53-independent apoptosis is activated when microtubule depolymerization during the mitotic G2/M transition is perturbed [130]. Different mechanisms for activating apoptosis are exploited by standard anticancer therapies: radiotherapy and cis-platinum induce apoptosis by damaging DNA [131], and taxol-based drugs induce apoptosis by stabilizing microtubules during mitosis [130].

Whereas normal cells rely primarily on oxidative mitochondrial respiration, most cancer cells use anaerobic metabolism. This classical observation of Warburg's led him to claim that '*Cancer cells originate from normal body cells in two phases. The first phase is the irreversible injuring of respiration.* (…) *The irreversible injuring of respiration is followed, as the second phase of cancer formation, by a long struggle for existence by the injured cells to maintain their structure, in which a part of the cells perish from lack of energy, while another part*

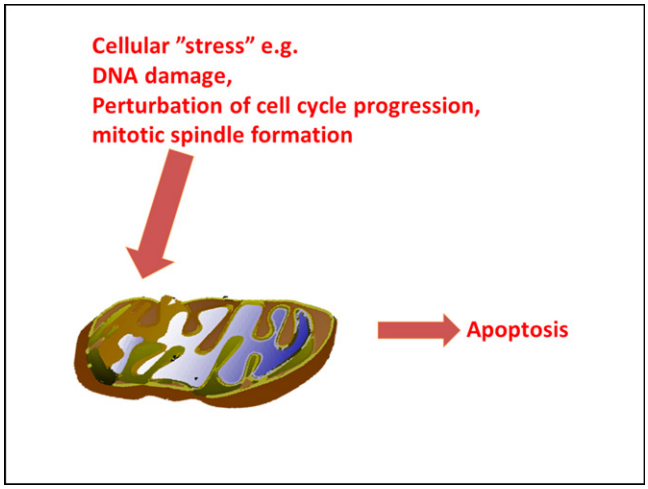

Figure 5. The activation of apoptosis by cancer stresses. A picture imported from wikipedia was used in this figure. (The following image imported from wikipedia was used in this figure: https://bg.wikipedia.org/wiki/%D0%9C%D0%B8%D1%82%D0%BE%D1%85%D0%BE%D0%BD%D0%B4%D1%80%D0%B8%D1%8F#/media/File:Mito.png (public domain).)

succeed in replacing the irretrievably lost respiration energy by fermentation energy' [132].

Later he clarified that statement by observing that the respiration of cancer cells is small relative to the consumption of glucose, but not small relative to the respiration of normal cells [133]. This is the Warburg hypothesis of cancer, and several recent studies confirm that mutations leading to the Warburg effect play an important role in malignant transformations.

Tumor cells have been shown to express only the embryonic isoform of pyruvate kinase. Switching pyruvate kinase expression to the adult isoform leads to the reversal of the Warburg effect and a reduced ability to form tumors in nude mice [134]. Deletion of embryonic pyruvate kinase accelerates mammary tumor formation in mice, where both embryonic and adult pyruvate kinases are inactivated in replicating mammary tumor cells [135]. Recent studies also suggest that mutations of p53 are associated with the Warburg effect [136].

The Warburg effect is puzzling because oxidative phosphorylation generates up to 36 ATPs from one glucose molecule, whereas the anaerobic process generates only 2. How can cancers grow rapidly while using a less efficient mechanism to generate energy? One possible explanation is that inefficient ATP production is a problem only when resources are scarce. Heiden *et al* suggested that proliferating cells may be adapted to facilitate the incorporation of nutrients into biomass and that the anaerobic state is more efficient for this purpose [137]. They argue that during growth, glucose is also a source of carbons for molecular synthesis and generating biomass. Oxidative respiration is a less efficient way of producing biomass, for some carbons are incorporated into useless Co_2. Some glucose must be diverted for macromolecular precursors such as alanine, ribose, or acetyl-CoA. They conclude that anaerobic is more efficient than aerobic metabolism at incorporating nutrients into biomass. This idea is supported by the fact that rapidly dividing microbes do not often depend on oxidative respiration even when they have access to oxygen. According to this explanation, the Warburg effect is not the direct cause of malignant transformation as Warburg suggested, but it does lead to the increased fitness of cancer cells by providing them with more carbon-based molecules with which to build cellular structures.

Recent studies suggest that the Warburg effect is also involved in the reverse aging of cancer cells and the development of cancer stem cells. The concept that cancer cells arise from embryonal-like stem cells was first proposed in the nineteenth century. The progress in oncology led to the discovery of aggressive pluripotent cancer stem-like cells [138]. Such cells have the key stem cell properties, including self-renewal, which drives tumorigenesis and aberrant differentiation into other cancer cells [139, 140]. Such cells are also more resistant to chemotherapy than other cancer cells and it has been shown, as well, that a high proportion of stem cells signifies a worse prognosis [140].

Recent studies indicate that the metabolic reprogramming leading to the Warburg effect plays a significant role in the 'reversed aging' and the development of cancer stem-like cells (cancer stem cells) [138]. Classical observations indicate that the Warburg effect is evident during the early stages of mammalian development. At the beginning of organogenesis, the embryo shows a high rate of anaerobic glycolysis, typical of the Warburg effect. Later, a metabolic shift toward oxidative respiration takes place [141]. In recent years, significant progress in embryology has been achieved as a result of the methodology discovered for reprogramming somatic cells into pluripotent embryonal-like stem cells [142]. It would appear that the stimulation of aerobic glycolysis

(Warburg effect) favors induced pluripotency [138]. Recent studies show similar phenomena in cancer stem cells. Glucose in the environment induces a significant increase in the percentage of stem-like cells in the overall cancer cell population. Interestingly, hypoxia can lead to the development of cancer stem cells and cause some therapeutic approaches to backfire [128], as shown by Conley *et al* [143], who generated intratumoral hypoxia in human breast cancer xenografts. The antiangiogenic agents, Sunitinib and Bevacizumab, were found to increase the population of aggressive cancer stem cells.

It has also been shown that the enhanced activity of the hypoxia-inducible factors, HIF-1α and HIF-2α, play crucial roles in the development of the stemness phenotype. The activated HIFs may induce both the glycolysis and pluripotency-associated transcription factors (for a review, see [144]). This suggests that cancer stem cells may have a selection advantage when cells cannot use oxidative cellular respiration.

As already mentioned, immortality is one of the traits of a cancer cell. Recent studies suggest another plausible explanation of the Warburg effect. A drug candidate**,** dichloroacetate, shifts metabolism from glycolysis to glucose oxidation and induces apoptosis [145]. Thus, the Warburg effect may help cancer cells inhibit apoptosis (see [146]). We should consider an apoptotic explanation of the Warburg effect in which malignant transformation leads to an inhibition of apoptosis and additionally shifts metabolism from glycolysis to oxidative respiration.

6.2. Cancer, neuronal aging, and apoptosis

As mentioned in chapter 5, neurodegenerative diseases are caused by the aging process of neuronal cells.

Patients with a history of cancer have experienced a reduced risk of Alzheimer's and Parkinson's diseases [147–150]. This observation raises the question, do some mechanisms link cancer to the aging of neurons?

One possible explanation is based on the differences in the metabolism of cancer and neuron cells. In contrast to cancer cells, the predominant mode of energy production in neurons is oxidative respiration. Two important factors in the early pathology of neurodegenerative diseases are mitochondrial dysfunction and oxidative stress. These lead to the creation of the 'inverse Warburg hypothesis,' according to which the regulation of mitochondrial respiration would compensate for the mitochondrial dysfunction that occurs during pathological aging. Studies supporting this idea show that an early marker of neurons susceptible to Alzheimer's disease is an increase in mtDNA and in levels of the cytochrome oxidase-1 protein. This compensatory change enhances production of the free radicals thought to cause metabolic perturbations (see [151]). I suggest that the 'inverse Warburg effect' could also be explained by a direct link between mitochondrial metabolism and apoptosis in which enhanced mitochondrial respiratory activity leads to pathological apoptosis and the degeneration of neurons. As already mentioned, a link between mitochondrial metabolism and cancer exists in the 'Warburg effect' where anaerobic respiration inhibits apoptosis. It is well known that apoptosis is involved in the pathology of both Alzheimer's and Parkinson's diseases; there are hallmarks of apoptosis in neuron cells that are dying [152, 153].

7. Conclusions

Apoptosis is an ancient mechanism whose pathways are shared by lineages with ancient common ancestors. We do not yet understand why apoptotic mechanisms evolved and were maintained in unicellular organisms. Mitochondria play an important role in apoptosis, for mitochondrial metabolism, respiration, and apoptotic mechanisms are functionally connected by cytochrome c and the AIFs (AIFs), which are involved in mitochondrial respiration. That the mitochondrial respiration of cancer cells is small relative to the consumption of glucose—the Warburg effect—suggests that the origin of apoptosis contains clues to the understanding of intrinsic tumor-suppressor mechanisms. Mitochondrial activity is crucial to tumor suppression by apoptosis and to mitochondrial respiration. There is also a link between pathological aging during both Alzheimer's and Parkinson's diseases and mitochondrial activity. Pathological aging occurs in neurons, which rely on oxidative respiration. According to the 'inverse Warburg hypothesis,' the mitochondrial activity is involved in the pathological process including pathological apoptosis in aging cells.

Several testable hypotheses follow from this view:

The ancient functional connection between respiration and apoptosis indicates it is likely that the deletion of apoptotic factors in hypoxia is beneficial to unicellular organisms.

The altruistic suicide hypothesis can be tested using experimental evolution. This hypothesis implies that competition between closely and remotely related unicellular organisms would have had an impact on the evolution of apoptotic traits. The competition between closely related conspecifics will favor the maintenance of apoptotic mechanisms. In contrast, competition between remotely related conspecifics will favor their weakening.

The function of apoptosis in cellular aging is not clear. I hypothesize here that apoptotic mechanisms participate in the removal of the toxic remnants of aged cells (cf the groups observed by Durand and described above). This can be tested experimentally by comparing the toxicity of the remnants of cells aging due to programmed and accidental cell death and could be particularly important in cases of neurodegenerative disease.

As already mentioned, there have been many studies testing the hypothesis developed from Warburg's. It has been shown that the stimulation of anaerobic glycolysis often enhances the tumorigenic phenotype. More research is still required.

On the other hand, there is little work testing the 'inverse Warburg hypothesis.' If cellular aging is reverse tumorigenesis, then the stimulation of anaerobic glycolysis will protect neuronal cells during the progress of a neurodegenerative disease. This hypothesis should be tested in depth. The hypothesis also suggests that diet will have an impact on the development of neurodegenerative diseases in animal models. Diets enriched in glucose should stimulate the glycolysis of neuronal cells and postpone pathological aging, and hypoxic conditions should have a beneficial effect on neurodegenerative diseases.

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