ADVANTAGES AND DISADVANTAGES OF APOPTOSIS IN THE AGING PROCESS

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Abstract: Researchers cannot predict as yet how long a human being can live. Life expectancy has been steadily increasing in the last century, but perhaps not always the quality of life in parallel with it. Future generations will be faced with the problems of an increased life expectancy along with the emergence of new age-related diseases. A deeper understanding of the aging process is crucial to ameliorate, if not to prevent, these projected new old-age diseases. One of the mechanisms responsible for healthy aging is through the effective maintenance of physiological, biochemical, and immunological functions. To carry this out, the organism needs to create new cells to replace old ones and to induce the disappearance of old and damaged cells. Apoptosis is involved in all these processes. However, if apoptosis is dysregulated, premature senescence-associated diseases are likely to appear. In our review, the focus will be on a better understanding of the role of apoptosis in the aging process. These signaling pathways will most assuredly be pharmacologically targeted in antiaging medicine therapies.

INTRODUCTION

Aging is a multifunctional and progressive loss of physiological, biochemical, and immunological functions that leads to a decreased survival rate and an increased risk of chronic diseases and disabilities.[1, 2] Aging is the main risk factor for chronic pathologies.[3] In addition, the extra years of life expectancy lead to an increased risk of multimorbidity.[4] An important issue arising from our increased understanding of the molecular mechanisms of aging is how to translate this knowledge into therapeutic approaches that delay aging and prevent chronic age-related pathologies.[5]

Although aging is a complex process, several molecular and cellular changes, such as metabolic dysfunction, dysfunctional mitochondria, diminished proteostasis, genomic instability, loss of stem cell regenerative capacity, increased persistent inflammation, generation of damaging reactive oxygen species (ROS), cellular senescence, telomere erosion, and chromatin and epigenetic alterations, have been reported as contributing to the aging process and aging phenotypes.[1,2] Interestingly, all of these mechanisms of aging can modulate the programmed cell death caused by apoptosis. As a consequence of intracellular or extracellular damage, apoptosis is activated as an adaptive response for the maintenance of homeostasis. At physiological levels, apoptosis promotes the morphogenesis and proper development of tissues and organs.[6] In the adult, apoptosis contributes to the elimination of nonfunctional damaged cells. Therefore, its dysregulation has an essential role in the development of age-related pathologies.[7] In this review, we will focus on both how apoptosis contributes to the aging process and how different aging hallmarks modulate cell death.

MOLECULAR MECHANISMS OF AGING

Dysfunction of signaling pathways associated with metabolism promotes the risk of energy imbalance. Energy deficiency extends life span in various species through downregulation of two proaging pathways: (1) the mTORC1/S6K1 pathway, which is activated by anabolic signals in response to high amino acid concentrations that induce protein synthesis and proliferation and inhibit autophagy; and (2) the insulin/IGF-1 pathway, which regulates glucose metabolism and body fat distribution through activation of mTOR and inhibition of AMPK. However, energy deficiency promotes the upregulation of two antiaging pathways: (1) the AMPK pathway, which is stimulated by increased levels of AMP/ADP caused by ATP depletion, leading to the activation of SIRT1, ULK1, nuclear factor erythroid 2–related factor 2 (Nrf2)/SKN-1, FOXO/DAF-16, the p53 pathways, and the inhibition of CRTC1, mTOR, and NF-κB; and (2) SIRT1, a sensor of increased levels of NAD+ that functions as a NAD+-dependent class 3 histone deacetylase and activates important proteins associated with stress response, metabolism, and survival, such as LKB1/AMPK, PGC-1α, FOXO1, and FOXO3.[1, 2, 8]

Dysfunctional mitochondria and the generation of damaging ROS contribute to aging and age-related diseases. Mitochondria and energy metabolism induce several signaling pathways, including apoptosis, autophagy, necrosis, stress response, and calcium homeostasis. Proaging pathways contribute to mitochondria dysfunction because increased energy availability (glucose), mitochondrial metabolism (ATP production), and respiration rate (ROS production) inhibit AMPK and SIRT1 antiaging pathways, with the consequent impairment of antioxidant enzymes (e.g., SOD and catalase) and PGC-1α activity.[1, 2, 9]

Increased oxidative damage and dysregulated stress response promote the accumulation of oxidatively damaged biomolecules and decrease energy efficiency. During aging, levels of ROS increase, while levels of antioxidant defense and proteins, such as CREB, NF-κB, and Nrf2, which mitigate cellular stress and eliminate or repair damaged molecules, decrease.[1, 2, 10] Although ROS levels increase with age, ROS are

able to promote cell survival, senescence, or death, depending on their level and the cellular environment. For example, the major lipid peroxidation product 4-hydroxynonenal (4-HNE) at low levels contributes to cell signaling that promotes gene expression, increases cellular antioxidant capacity, and exerts an adaptive response; at medium levels, it causes cell damage, leading to the induction of autophagy, senescence, or cell cycle arrest; and at high or extremely high levels, it stimulates protein and/or DNA adducts, leading to apoptosis or necrosis cell death, respectively.[11]

Dysregulated Ca2+ levels contribute to aging because continued increases in Ca2+ promote oxidative damage, metabolic stress, mitochondrial dysfunction, and apoptosis. In neurons, physiological levels of Ca2+ are essential for modulating synaptic transmission, synaptic plasticity, and survival, but an uncontrolled increase of Ca2+ activates phospholipases, endonucleases, and Ca2+-sensitive proteases such as calpains, promotes caspase-mediated apoptosis, stimulates PARP1-mediated cell death, and decreases expression of BDNF, which leads to age-related neurodegeneration.[1, 12]

Diminished proteostasis promotes the formation of misfolded and abnormal protein aggregates since the capacity to eliminate damaged and dysfunctional proteins and organelles is decreased. Chaperones (e.g., HSP60, HSP70, and HSP90) contribute to protein folding, refolding, disaggregation, oligomeric assembly, trafficking, and degradation that prevent protein misfolding and abnormal protein aggregation. If folding occurs with errors, chaperones promote protein degradation by the ubiquitin–proteasome system or by the autophagy–lysosome system.1, 2, 13 Proaging pathways (insulin/IGF-1 and mTOR) decrease proteostasis through inhibition of autophagy, while antiaging pathways (AMPK and SIRT1) increase proteostasis by activating autophagy.

Increased persistent inflammation promotes the aging process because the upregulated proinflammatory pathways (e.g., NF-κB, p38 mitogen-activated protein kinase (MAPK), IL-6, JAK/STAT, and PI3K) and mediators of inflammation (e.g., interferon γ, tumor necrosis factor alpha (TNF-α), growth factor, ROS, and hydrolytic enzymes) contribute to dysfunction of the immune system (decreased adaptive and innate immune response), increase cellular senescence phenotype, and decrease autophagy. On the other hand, the accumulation of damaged macromolecules and cells, harmful products that are produced by the microbiota, mitochondrial dysfunction, and cellular senescence, promotes age-associated chronic inflammation or "inflammaging," which leads to the aberrant activation of immune cells, resulting in immunosenescence.[1, 2, 14]

Cellular senescence is the irreversible arrest of cell proliferation (growth) as a consequence of persistent telomeric, genomic, or epigenetic damage; oncogenic or uncontrolled mitogenic signaling (e.g., activation of MAPK, growth factor receptors, chronic stimulation by cytokines, and loss of PTEN); or aberrant activation of tumor suppressive pathways (e.g., p53/p21 and p16INK4a/pRB pathway).14 In young organisms, senescence prevents the proliferation of damaged cells and encourages the optimal repair of damaged tissue. Meanwhile, the immune system eliminates senescent cells that produce a proinflammatory senescence-associated secretory phenotype (SASP). However, in the case of chronic senescence, the production of a less proinflammatory SASP or the age-related deficiency of the immune system makes elimination of senescent cells difficult, resulting in increasing age-associated degenerative phenotypes.[2, 14] Depending on the cell type, senescence can be replicative (when mitotic somatic cell division ends), premature (when mitotic cells are exposed to stress), or postmitotic (when a mature nondividing cell undergoes cellular senescence).[15]

Telomere erosion contributes to aging because the telomere is essential in preserving the integrity of the genome by avoiding chromosomal degradation and short chromosomal ends. For the human telomere, length positively correlates with protection against age-related diseases, better cognitive functions, improved lipid profiles, and healthy aging, along with exceptional longevity. Moreover, progressive and cumulative loss of the telomere leads to replicative senescence or apoptosis, while its ectopic expression confers immortality on mortal cells. Most normal somatic cells have low telomerase activity, while human cancer cells have a high telomerase activity.[2, 16]

Compromised DNA damage repair mechanisms contribute to aging, age-related diseases, and genomic instability. The impairment of DNA damage repair mechanisms, such as mismatch repair, base excision repair, single-strand break repair, nucleotide excision repair, homologous recombination, or nonhomologous end-joining, promotes cell death by apoptosis, cellular senescence, or reduced stem cell function.[1, 2, 17]

Loss of stem cell regenerative capacity contributes to the aging process because the cell is not able to self-renew and maintain tissue homeostasis after damage or injury, which leads to decreased proliferation and increased senescence and apoptosis. During development and reproduction, stem cell activity is high, but with age this activity declines in most tissues. However, excessive proliferation of stem cells can lead to exhaustion and premature aging.[1, 2, 18]

Chromatin and epigenetic factors affect gene expression dynamically by regulating the access of transcriptional machinery to DNA. In the context of aging, it has been reported that as a consequence of environmental stimuli and glucose sensing, Ras/AC/PKA, mTOR, AMPK, SIRT, and the insulin/IGF-1 pathway, the dysregulation of intracellular metabolites promotes epigenetic changes, such as loss and posttranslational modification of histones, transcriptional deregulation because of epigenetic change, reorganization of heterochromatic domains, alteration of DNA methylation levels and patterns, chromatin remodeling, and breakdown of the nuclear lamina, which dynamically contribute to the aging process.[2, 19]

APOPTOSIS IN BRIEF

In brief, it is possible to induce apoptosis by extrinsic and intrinsic pathways. Intrinsic (or mitochondrial) apoptosis is induced after cellular stress/damage, and includes inactivation of antiapoptotic proteins (e.g., Bcl-2) and the production of ROS and ceramide in mitochondria, which promotes release into the cytosol of proapoptotic proteins (e.g., Bax, cytochrome c, apoptosis-inducing factor, endonuclease G, and second mitochondria-derived activator of caspases/direct inhibitor of apoptosis (IAP)-binding protein with low pI (Smac/Diablo)), activation of the caspase activator apoptotic protease activating factor 1 (APAF-1) and caspase-9, formation of the apoptosome, and activation of caspase-3, -6, and -7. Extrinsic apoptosis can be activated through the death receptors family of proteins, such as CD95 (Fas/Apo1), TNF-related apoptosis-inducing ligand (TRAIL) receptors, and TNF receptors, which leads to induction of the same caspase cascade as that of the intrinsic pathway. Apoptosis can be inhibited by several proteins, such as XIAP, cIAP1, cIAP2, survivin, livin (ML-IAP), NAIP, Bruce (apollon), and ILP2. Moreover, efficient DNA repair machinery, activation of autophagy, heat shock proteins, and ER stress and the unfolded protein response (UPR) inhibit apoptosis and promote cell survival. Complete descriptions of apoptosis are available in excellent reviews (e.g., see Refs. [20] and [21]). The cellular response to damage is summarized in Figure 1.

MECHANISM OF APOPTOSIS DYSREGULATION IN AGING

Metabolic regulation and apoptosis

Downregulated mTOR signaling under certain stimuli and in specific cell environments can stimulate apoptosis in some postmitotic cells (e.g., cardiomyocytes[22]) and some mitotic cells (e.g., endothelial progenitor cells[23]). In this context, downregulation of mTOR contributes to longevity by removing damaged cells, improving homeostasis, and inducing apoptosis to eliminate senescent cells accumulated during aging. However, aberrant downregulation of mTOR and upregulation of apoptosis in nonsenescent mature cells, which are unable to undergo mitosis or cell division (postmitotic cells), may lead to increased cell loss, tissue dysfunction, and exacerbated postmitotic cell-associated pathological conditions. Under certain conditions, such as cardiac ischemia/reperfusion (I/R) injury, the increased activity of mTOR helps to prevent uncontrolled stimulation of apoptosis or autophagy, decreasing cardiomyocyte death, and preserving cardiac function.[24] Interestingly, it has been reported that in tubular epithelial and mesangial cells, the depletion of mTOR reduces apoptosis.[25]

Insulin/IGF-1 is another proaging pathway with antiapoptotic effects, by promoting the activation of the RAF/MEK/ERK and PI3K/Akt pathways. Moreover, IGF-1 modulates apoptosis through several signaling pathways. For example, in some postmitotic cells, such as neurons, IGF-1 protects them from apoptosis by upregulating the antiapoptotic protein Bcl-2 and decreasing caspase-3 activity.[26] In addition, netrin-1 protects cortical neurons from apoptosis by activating the ERK signaling pathway.[27] In this context, we suggest that in response to certain cell damage, insulin/IGF-1 inhibits apoptosis to control tissue destruction and contributes to cell survival, proliferation, damage repair, and regeneration. However, oxidative cell death in neurons is associated with the upregulation of ERK.[28] Moreover, it has also been reported that insulin/IGF-1 activates PI3K/Akt, which in turn activates mTOR and results in the stimulation of NF-κB, an important modulator of inflammation with both proapoptotic and antiapoptotic effects, depending on the cellular context.[29] In this case, it could be possible that under certain conditions, aberrant activation of RAF/MEK/ERK contributes to preventing the proliferation of damaged mitotic cells or the accumulation of senescent postmitotic cells.

In response to nutritional stress, AMPK can promote gene expression to increase energy production in cells or promote apoptosis. Prolonged activation of AMPK induces apoptotic cell death by increasing the activity of JNK, p53, FOXO3, caspase-3/7, PARP, the Bax/Bcl-2 ratio, and by decreasing the activity of mTORC1 and the antiapoptotic protein Bcl-2.[30, 31] However, modest activation of AMPK inhibits apoptosis by decreasing cleaved caspase-3 protein expression and caspase-3/7 activity.[32] It is possible that apoptosis induced by prolonged activation of AMPK preserves homeostasis by preventing accumulation of mitotic senescent cells during aging, inducing loss of damaged postmitotic cells, and by limiting tissue dysfunction. On the other hand, inhibition of apoptosis by moderate activation of AMPK may prevent pathological conditions resulting from aberrant activation of apoptosis during cell injury/damage.

In response to different stress conditions (e.g., nutritional, catabolic, mechanical, or oxidative), SIRT1 inhibits apoptosis, increasing cell survival and life span through deacetylation of important apoptosis-inducible nuclear proteins, such as p53, the forkhead box class O (FOXO) family of proteins, Ku70, and the RelA/p65 subunit of NF-κB, which leads to the inhibition of caspase-3/9, reduction of Bax, and upregulation of Bcl-2.[33] FOXO3 plays a key role in promoting longevity, predominantly in response to stress, by inducing apoptosis through stimulation of death receptor ligands such as Fas ligand and TRAIL, and induction of Bin and PUMA (transcriptionally upregulated by p53).[34] This antiapoptotic effect of SIRT1 can help in protecting organs from apoptosis during tissue injury and dysfunction. However, under

certain conditions, this antiapoptotic effect of SIRT1 is unfavorable because it promotes oncogenic transformation. Interestingly, it has been reported that in specific types of cancer, for example, human chondrosarcoma, SIRT1 has a proapoptotic effect and promotes deacetylation of p65 subunit of NF-κB complex and the activation of caspase-3.[35]

Mitochondria, oxidarive stress, autophagy and apoptosis

It is known that high levels of oxidative stress promote both damage of biomolecules and cell death through the mitochondrial apoptosis pathway.[21] Physiological levels of oxidative stress and transient reduction of mitochondrial function lead to increased endoplasmic reticulum (ER) stress and induce a UPR to promote cell survival and longevity. In fact, pharmacological approaches that increase the life span (e.g., resveratrol and rapamycin) induce the activation of the UPR,[36] but under high oxidative stress levels, the cell is unable to control high levels of ER stress by the UPR and the cell undergoes apoptosis.[37] In addition, when energy metabolism decreases, apoptosis is inhibited by AMPK through SIRT1. Although high levels of ROS/reactive nitrogen species promote mitochondrial dysfunction, resulting in the subsequent depletion of ATP and apoptosis, low levels of ATP production in certain contexts can have advantages for cells by promoting survival and longevity. In fact, under reduced levels of ATP, for example, during nutrient starvation, antiaging pathways, such as AMPK and SIRT1, and biological processes such as autophagy are stimulated, while proaging pathways such as mTOR are inhibited. An aberrant loss of mitochondrial function is possibly associated with apoptosis, while the transient loss of mitochondrial function could be linked to survival and longevity. Indeed, some long-lived postmitotic cells (e.g., osteocytes) undergo apoptosis during the aging process due to decreasing connexin 43 (Cx43), which disrupts the PTEN/pAkt pathway and blocks the antiapoptotic effect of IGF-1.[38] Moreover, increased ROS, which is a hallmark of aging, promotes the activation of MAPKs, which then encourage the intrinsic apoptosis pathway by promoting mitochondrial dysfunction, ER stress, and Ca2+ overload, which can lead to osteoblast apoptosis.[39] Aberrant loss of mitochondria function and oxidative stress-induced apoptosis of skeletal muscle cells through NF-κB and FOXO have been implicated in sarcopenia, the age-related loss of skeletal muscle mass.[40] Paradoxically, apoptosis contributes to the removal of skeletal muscle fibers by eliminating single nuclei (and their portion of cytoplasm) of myofibers (multinucleated cells) during muscle atrophy.[41]

Although apoptosis and autophagy are stimulated to promote stress response, they have opposite effects on the aging process. Indeed, signaling pathways responsible for inducing apoptosis lead to an inflammatory phenotype that accelerates aging and promotes age-related pathologies, while signaling pathways that induce autophagy lead to increased survival and longevity. Moreover, accumulation of abnormal proteins and protein aggregates responsible for common age-related neurodegeneration involves a disturbance of autophagy that promotes mitochondrial dysfunction and induces apoptosis. Beclin-1, which is essential for the initiation of autophagy, is unable to induce autophagy when it is cleaved by caspase. However, the C-terminal fragment promotes the release of cytochrome c from mitochondria, resulting in apoptosis induction. For example, spermidine, an autophagy inducer, suppresses caspase-3 activation and inhibits Beclin-1 cleavage and the release of cytochrome c from mitochondria, which leads to diminished neuronal cell damage.[42] Interestingly, while apoptosis induced by the proapoptotic protein Bax reduced autophagy by enhancing caspase-mediated cleavage of Beclin-1 in cells deficient for antiapoptotic capacity, Bax can promote autophagy in cells with sufficient antiapoptotic capacity through disruption of the Bcl-xL–Beclin interaction.[43] The complex interconnection between autophagy and apoptosis has also been elucidated in mesenchymal stem cells (MSCs). Here, the authors found that autophagy promotes the survival of MSCs under starvation but promotes apoptosis in an inflammatory microenvironment through inhibition of Bcl-2 and activation of caspase-3.[44] Usually, autophagy is a

protective antiapoptotic process. Indeed, autophagy decreases with age, and pharmacological and genetic restoration of the pathways responsible for autophagy activation can potentially delay aging and increase longevity in diverse organisms.[45]

Inflammation, senescence and apoptosis

The inflammation process promotes a concomitant increase of oxidative stress and mitochondria dysfunction that result in upregulation of apoptosis. It is known that uncontrolled mitochondrial dysfunction promotes senescence and cells become resistant to apoptosis. In this case, the induction of apoptosis has the advantage because it can eliminate senescent cells and promote regeneration and homeostasis. The inefficient elimination of dysfunctional T cells is the major cause of immunosenescence because apoptosis is essential to immune system development, along with adult immune homeostasis (e.g., elimination of self-reactive T and B cells and virus-infected cells, and diminishing effector T cells after an immune response).[46] However, an aberrant increase of apoptosis can promote immunosuppressive effects. It is possible that an increase in life span could be the result not only of an efficient immune system but also of an effective apoptosis machinery able to eliminate damaged immune cells.

Interestingly, mitochondrial function and oxidative stress are regulated by p53, which also has a key role in senescence. Activation of p53 in response to genotoxic or oxidative stress induces DNA repair, inhibits IGF-1 and mTOR, and promotes apoptosis.[47] A complete description of p53-induced apoptosis mechanisms has been provided by Chipuk and Green.[48] In brief, after activation, nuclear p53 directly modulates the expression of proapoptotic genes, for example, BAX, PUMA, NOXA, and BID. Cytoplasmic p53 interacts with antiapoptotic Bcl-2 proteins (Bcl-2 or Bcl-xL) and activates proapoptotic multidomain Bcl-2 proteins (Bax and Bak). The activation of p53 activity is essential because its low activity decreases apoptosis, causing uncontrolled proliferation and growth (e.g., cancer). However, high activity leads to acceleration of aging because of the increased senescent cells, which are resistant to apoptosis. Although the association of p53 with human aging is not well known, it has been reported that dysfunctional modulation of p53 by MDM2 results in an uncontrolled increase of p53 activity, which leads to deleterious effects on the human aging processes, including increasing premature senescence in fibroblasts.49 Likewise, other segmental progeroid syndromes, such as ataxia telangiectasia, Hutchinson–Gilford progeria, and Cockayne syndrome, show high activation of the p53 pathway.[49] Besides p53, the NF-κB pathway induces resistance to apoptosis by promoting senescence phenotypes. NF-κB, which is upregulated in aging, can be activated by proaging pathways (e.g., insulin/IGF-1 or mTOR) and inhibited by AMPK, SIRT1, and FOXO. NF-κB also induces an inflammatory response, which is essential for repair and regeneration, but an aberrant inflammatory response induces cell dysfunction, tissue degeneration, accelerated aging, and death. NF-κB can inhibit apoptosis by upregulating the expression of antiapoptotic survival genes, such as Bcl-xL and IAPs, and by inhibiting stress-activated protein kinases, such as JNK, which stimulate apoptosis through TNF-α and ROS.[50]

Telomerase loss, dna damage and apoptosis

Over-expression of human telomerase reverses trancriptase (hTERT) in mitotic cells (e.g. CHO K1) induces proliferation and promotes resistance to apoptosis [51]. Moreover, in response to injuries, hTERT protects postmitotic cell (neurons) from apoptosis [52]. Given that telomerase activity is essential to promote cellular resistance to apoptosis, inhibition of hTERT induces apoptosis. In fact, it has been reported that inhibition of hTERT induces mitochondrial-dependent pathway through upregulation of Bax and cleaved caspase-3/9, and downregulation of Bcl-2 [53]. Although the molecular mechanisms that determine whether telomere damage leads to senescence or apoptosis remains unclear. Recently it has been reported that association between p16, a cyclin-dependent kinase inhibitors inductor of cell cycle arrest and senescence,

and caspase-3 lead to senescence induction by inhibiting caspase-3 activation and apoptosis, because telomerase instability encourages telomere damage-dependent caspase-3 activation and apoptosis, but not senescence, in p16-deficient cells. The authors suggest that p16 has a direct role in telomere damage-dependent senescence by limiting apoptosis via binding to caspase-3 [54].

It is known that in both cancer and non-cancer cells, induction of DNA-damage promotes apoptosis. Indeed, several common anti-cancer drugs such as doxorubicin (DOX) increase DNA damage, which lead to cell cycle arrest and finally apoptosis through activation of p53/PUMA or Bim [55]. Usually, mTOR is upregulated in cancer. Aberrant increase of mTOR, as a result of TSC2 or PTEN loss, promote DNA damage and apoptosis through CREB1 in autophagy-defective cells, and mTOR inhibition-mediated chemoresistance lead to inhibition of CREB1 through activation of autophagy [56]. Although its role in protein degradation, autophagy protects hematopoietic cells from nuclear radiation exposure by promoting the elimination of ROS, blocking apoptosis and regulating DNA damage response components e.g. DNA ligase-4, Ku80, XRCC4, BRCA1 and p95/NBS1, probably through degradation of proteins that inhibit the expression of the DNA damage repair proteins, or through inhibition of ubiquitin-proteasomal degradation of the DNA damage repair proteins [57].

Stem cell and apoptosis

The use of stem cell therapy to repair and promote regeneration of damaged tissue is presently a growing area of research. However, the main problem is that the aggressive microenvironment generated by tissue damage and proinflammatory modulators makes transplanted stem cells extremely sensitive to apoptosis. In this context, promoting survival by inhibiting apoptosis in stem cells is a good option. For example, neural stem cells (NSCs) are critical for normal neurogenesis because they differentiate into neurons and glial cells. It has been reported that ketamine-induced neuroapoptosis leads to reduced neurogenesis and cognitive impairment. Treatment with dexmedetomidine protects NSCs from ketamine-induced injury through reduction of apoptosis by activating the PI3K/Akt/GSK-3β signaling pathway.[58] The survival of MSCs in response to nutrient deprivation–induced apoptosis was improved by cyclic helix B peptide through mechanisms involving the activation of the Nrf2/SIRT3/FOXO3a pathway, improving mitochondrial dysfunction and preventing apoptosis.[59] Adrenomedullin protects MSCs from hypoxia and serum deprivation–induced apoptosis through upregulation of pAkt and pGSK3β, activation of antiapoptotic Bcl-2 signaling pathways, and downregulation of the activation of caspase-[3.60] However, in the context of cancer, patients will benefit from promoting apoptosis in cancer stem cells, which are considered the main cause of cancer recurrence. A set of molecules that can suppress cancer cell proliferation by inducing apoptosis has been reported. For example, quercetin-3-methyl ether induces apoptosis by inhibiting the expression of NOTCH1 and inducing the phosphorylation of PI3K and Akt.[61] Curcumin also decreases cell proliferation and promotes apoptosis in liver cancer stem cells through upregulation of caspase-3/9 and Bax, downregulation of Bcl-2, and inhibition of the PI3K/Akt/mTOR signaling pathway.[62]

Epigenetic and apoptosis

Lysine deacetylases (HDAC (Zn2+-dependent) and sirtuins (NAD+-dependent)) eliminate the acetyl group from the lysines of histones and nonhistone proteins, which, for example, can promote epigenetic regulation and protein stability and activity. These deacetylases are essential to detect DNA damage and contribute to DNA repair.[63] In the context of cancer, where most of the HDACs are upregulated, inhibition of HDAC promotes apoptosis through different mechanisms. For example, HDAC inhibitors stimulate extrinsic apoptosis pathways by activating caspase-8/10 through death receptors such as Fas (Apo-1 or CD95), TNF receptor 1, TRAIL receptors (DR4 and DR5), DR3 (Apo3), and DR6. Moreover,

HDAC inhibitors stimulate the intrinsic apoptosis pathways by increasing proapoptotic Bcl-2 proteins (Bid, Bad, and Bim) and decreasing antiapoptotic Bcl-2 proteins (Bcl-2, Bcl-xl, and Mcl-1).[64] Interestingly, a new link between HDACs, autophagy, and apoptosis has been reported in postmitotic cells after injury. The authors found that valproic acid, an inhibitor of HDACs, protects neurons against traumatic brain injury–induced death through inhibition of HDAC3 expression; upregulation of antioxidative factors (Nrf2/ARE), histone H3/H4 acetylation, and autophagic markers (LC3-II, Beclin, ATG3, and ATG7); downregulation of inflammatory factors (TNF-α, IL-1β, and IL-6); and reduction of apoptotic factors (cleaved caspase-3 and Bax).[65]

EXTRAORDINARY AGING AND APOPTOSIS

Some evidence shows that apoptosis is downregulated during aging, but improves considerably in people of extraordinary age (centenarians). For example, Fas and FasL are essential modulators of immune system apoptosis that promote clearance of stimulated T cells in order to avoid chronic inflammation and age-related inflammatory pathologies. It has been reported that sFas (an IAP) increases, while sFasL (a stimulator of apoptosis) and total cytochrome c (released from cells during apoptosis) decrease, in serum, which contributes to a global apoptosis balance that declines with age.66 Apoptosis in centenarians has been examined in several reports. The authors found by analysis of different forms of Fas and FasL in a group of centenarians that excessive lymphocyte apoptosis is prevented in physiological conditions but they have an enormously efficient Fas/FasL apoptotic pathway, inducible after stimulation with anti-CD3 mAb and TPA plus ionomycin.[67] Functional transcriptomic analysis of peripheral blood mononuclear cells showed that apoptosis is maintained better in centenarians (extraordinary aging) than in septuagenarians (normal aging). Interestingly, the authors found that Bcl-xL, an inhibitor of intrinsic mitochondrial apoptosis pathways, is upregulated in centenarians, whereas cytochrome c, a marker of intrinsic apoptosis, was downregulated in centenaries, suggesting that Bcl-xL is essential to successful aging and protection against age-associated damage. Moreover, other markers of healthy aging, such as mitochondria membrane integrity, lymphocyte function, and stress response, were also maintained in centenaries, probably as a consequence of Bcl-xL upregulation.[68]

In an excellent review, Tian and colleagues summarized some pathways responsible for the differences in longevity between short- and long-lived species.[69] The authors showed that some molecular mechanisms, such as extraordinary cancer resistance, potent DNA damage/repair capacity, downregulation of IGF-1 levels in plasma or IGF-1R in brain, upregulation of telomerase binding proteins (e.g., Ku80) that protect telomeres from damage, as well as an efficient proteostasis network, contribute to longevity in long-lived species. Interestingly, since all these mechanisms can modulate apoptosis, we suggest that under physiological conditions upregulation of apoptosis increases loss of nonsenescent mature cells, tissue dysfunction, and promotes pathological conditions. However, after damage/injury, an effective apoptosis response contributes to increasing longevity; in fact, apoptosis decreases the accumulation of mutations that promote genomic instability and aging. Maximum life spans differ up to 30-fold between mouse and human and apoptosis could have a key role for the interspecies discrepancy in longevity. It has been found that expression of Ku80 and DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is higher in long-lived species (human) compared with short-lived species (mouse).[70] In response to stress (e.g., oxidative or genotoxic), DNA damage induces apoptosis, and because short-lived species have lower levels of DNA repair machinery, it could be possible that short-lived species trigger apoptosis in response to higher levels of stress than long-lived species, which can be more efficient in maintaining tissue homeostasis after damage.

EFFECT OF ANTI-AGING APPROACHES ON APOPTOSIS

Interestingly, some pharmacological and nonpharmacological antiaging interventions decrease age-related apoptosis in postmitotic cells, which is an advantage by preventing cell loss and tissue dysfunction, whereas in mitotic cells some antiaging interventions increase apoptosis, which is an advantage in avoiding accumulation of dysfunctional cells. For example, resveratrol inhibits apoptosis in gastrocnemius muscle and neurons,[71] caloric restriction inhibits apoptosis in cardiomyocytes [72] and neurons,[73] and exercise decreases apoptosis in gastrocnemius muscle and neuron cells.71 On the other hand, caloric restriction increases apoptosis in the liver.[74]

Innovative research proposes a new antiaging approach to target aged senescent cells by using apoptosis-activating agents.[75] These agents that specifically remove senescent cells by inducing apoptosis are called senolytic drugs. Some examples of senolytics include dasatinib (tyrosine kinase inhibitor) and quercetin (flavonoid), which induce apoptosis in senescent cells in vitro. Interestingly, these senolytics also promote apoptosis of senescent cells in vivo, leading to extended healthspan in mice. Molecular mechanisms of senolytics include targeting senescent cell antiapoptotic pathways. The molecular targets of dasatinib are dependence receptors/Src kinase/tyrosine kinase, while quercetin targets the Bcl-2 family, p53/p21/serpine, and PI3K/Akt.[75]

In mitotic cells with high proliferative capacity (cancer cells), antiaging interventions (e.g., resveratrol and metformin) promote cell death.[76] Antiaging interventions such as caloric restriction and exercise reduce global protein synthesis through AMPK-mediated phosphorylation and inhibition of eukaryotic elongation factor 2 (eEF2). Research in our laboratory has shown that in response to oxidative stress-mediated lipid peroxidation, eEF2 is modified and thereby contributes to the inhibition of global protein synthesis that occurs during aging, as well as encouraging selective translation of specific proteins that promote cell survival under oxidative stress conditions.[77] Diminished rate of protein synthesis under conditions of stress, for example, ER stress, decreases the load of substrates presented to the folding machinery in the ER lumen and promotes cell survival. For example, it has been reported that AMPK protects cardiomyocytes against ER stress-induced apoptosis during hypoxia via inhibition of protein synthesis through inactivation of eEF2.[78] We found that eEF2 is an important regulator of the synthesis of XIAP, an antiapoptotic protein able to inhibit activation of the caspase cascade.[79] These findings suggest that under oxidative stress conditions, eEF2 contributes to cell survival through different mechanisms, one of which is to prevent apoptosis.

CONCLUSION

Physiological and biochemical functions decrease with age and could be a major risk factor for the dysregulation of signaling pathways such as apoptosis. In fact, as we have summarized, all molecular mechanisms of aging discussed in this manuscript can regulate programmed cell death by apoptosis (Fig. 1 and Table 1). The advantages and disadvantages of apoptosis are summarized in Figure 2. In mitotic and postmitotic cells, low levels of apoptosis contribute to the accumulation of damaged cells, senescence, and genomic instability, but in response to injury/damage, low levels of apoptosis prevent tissue destruction and promote cell survival, proliferation, damage repair, and regeneration. In mitotic and postmitotic cells, physiological levels of apoptosis have several advantages for the aging process. In fact, elimination of dysfunctional cells and reduced accumulation of senescent cells improve homeostasis and longevity. A moderate increase of apoptosis in mitotic, highly proliferative cells improves cell turnover rates, whereas in postmitotic cells apoptosis contributes to cell loss and tissue dysfunction. In mitotic and postmitotic cells, high levels of apoptosis are a significant disadvantage because they exacerbate tissue damage and cell death.

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FIGURES

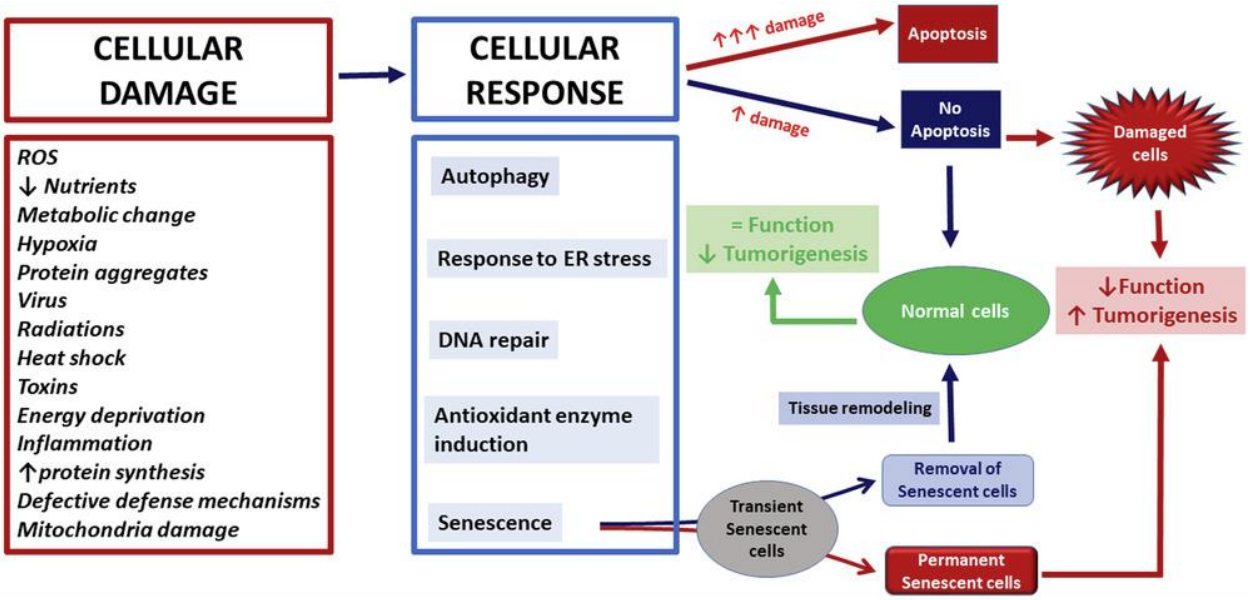

Figure 1. **Cellular response to damage.** Apoptosis is considered the ultimate result of severe rather than minor cellular damage, with the main goal being to get rid of undesired cells. Once the cells are subjected to a specific type of damage, the general and physiological sequence of events involves several biochemical mechanisms, depending on which tissue, type of cells, and subcellular organelles are involved, and if biomolecules become affected. All these mechanisms can basically be considered as cytoprotectives and include autophagy, response to ER stress, senescence, DNA repair, and antioxidant enzyme induction. Ideally, if the damage is not severe and these mechanisms are indeed efficient, the outcome would be cell survival and the ultimate reestablishment of cellular physiology and tissue function. But if the damage is severe and/or there are some failures in the cellular response mechanisms to mitigate the damage, a huge number of undesirable cells will accumulate, and the function of the tissue can be seriously compromised, leading to or aggravating several pathologies.

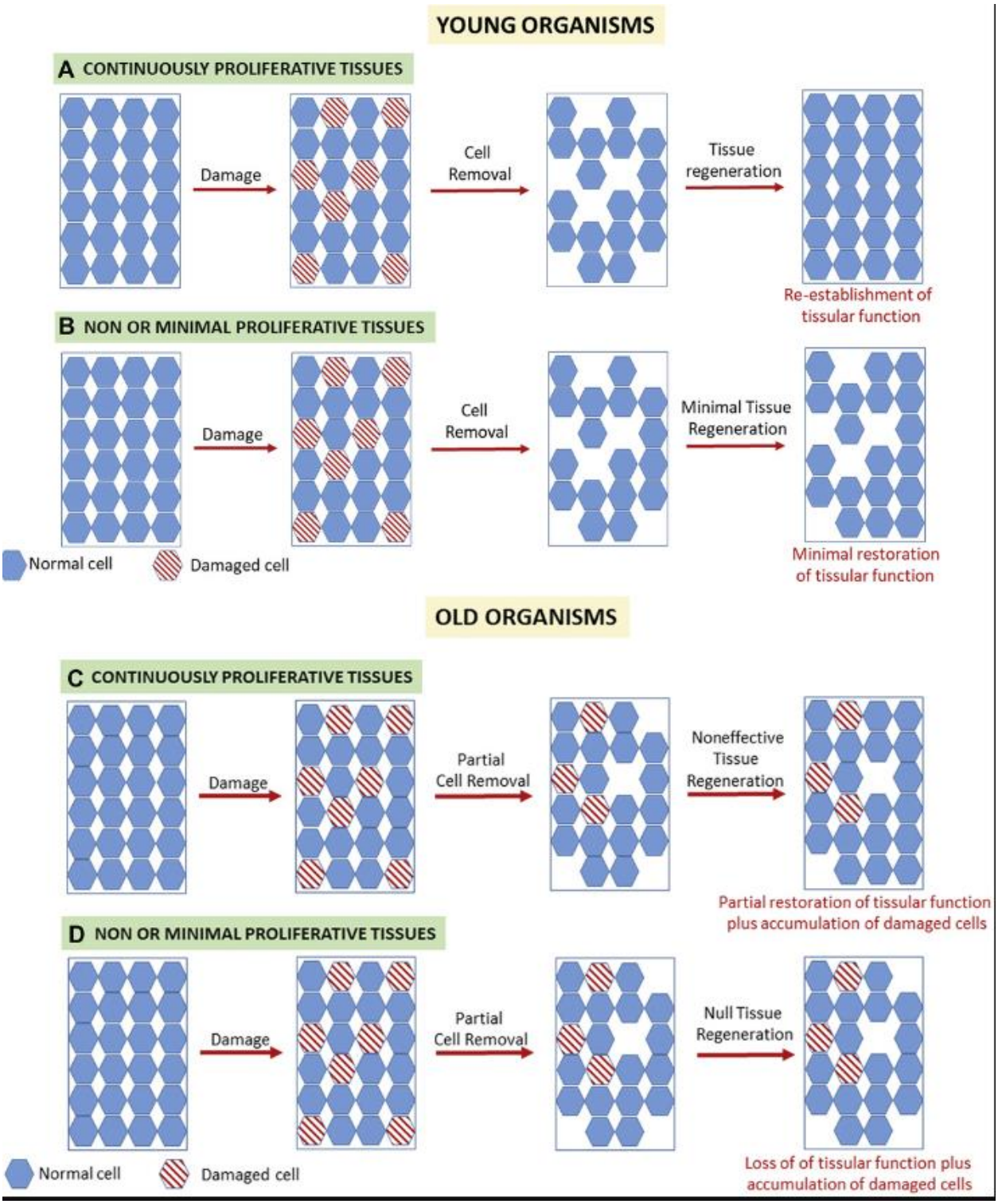

Figure 2. Advantages and disadvantages of Apoptosis. Advantages and disadvantages of apoptosis. Organisms continuously replace damaged cells as a part of a normal physiological program of maintaining tissue health. However, apoptosis can represent advantages or disadvantages, depending on whether the damage that causes apoptosis is transient or persistent, the type of tissue (whether or not the

cells frequently divide), and the degree of health of the individual and their age. The different types of damage that can cause apoptosis are: reactive oxygen species (ROS), lack of nutrients, hypoxia, protein aggregates, viruses, radiation, heat shock, toxins, energy deprivation, inflammation, increased protein synthesis, and defective defense mechanisms against these cellular insults. In terms of proliferative capacity, tissues in the body can be divided into three groups: (1) tissues whose cells continuously divide, for example, hematopoietic cells in the bone marrow and most surface epithelia, including skin, oral cavity, vagina, ducts, gastrointestinal tract, and others; (2) tissues whose cells divide regularly at certain times, for example, cells belonging to the parenchyma of most solid tissues, such as liver, kidney, and pancreas, and also endothelial cells, fibroblasts, and smooth muscle cells; (3) tissues whose cells do not proliferate, for example, neurons and cardiac muscle cells, although limited stem cell proliferation occurs in some areas of the adult brain. Also, there is evidence that heart muscle cells may proliferate after myocardial necrosis. Skeletal muscle cells are also classified into this group of cells, but satellite cells provide some regenerative capacity for muscle. In a young organism (A and B), which has a high regenerative potential, apoptosis after intense and persistent damage in tissues whose cells divide continuously (A) represents a safeguard mechanism to remove undesirable and senescent cells. These cells can be replaced from stem and progenitor cells and the function of the tissue can be reestablished. In young organism tissue with minimal proliferative capacity (B), excessive apoptosis would affect cellular and tissue activity since cells cannot be replaced. Nevertheless, this situation would be an advantage over that in which, due to the absence of apoptosis, the cell becomes cancerous. In old organisms (C and D), regenerative capacity is limited, in both continuously dividing tissues (C) and noncontinuously proliferative tissue (D). Although dead cells cannot be replaced, at least apoptosis would prevent the accumulation of senescent and damaged cells. Also, the problem is that apoptotic mechanisms as well as the response against damage fail with age. Therefore, the scenario in old tissues is a general loss of cell numbers along with an increased number of damaged cells with the tendency to become cancerous and cause or aggravate several pathologies.

Table 1. Signaling pathways that regulate both longevity and apoptosis.

Signaling Pathway	Activation level	Effect on Longevity	Apoptosis	Biological function
mTOR	↓	↑	↓	Promote autophagy under physiological conditions.
	↓↓	↓	↑	Promote non-senescent mature cells loss and tissue dysfunction under physiological conditions.
	↑	↓	↑	Decrease autophagy under physiological conditions.
	↑	↑?	↓[a]	Preserve postmitotic cells function after injury.
I/IGF1	↓	↑	↓	Promote autophagy under physiological conditions.
	↑	↓	↑	Decrease autophagy under physiological conditions.
	↑	↑?	↓[a]	Preserve postmitotic cells function after damage.
AMPK	↑	↑	↓	Promote autophagy under physiological conditions. Moreover, preserve cells function after injury.
	↑↑↑	↑?	↑[b]	Induce loss of damaged postmitotic cell and avoid increase of mitotic senescent cells after damage.
SIRT1	↑	↑	↓	Promote autophagy under physiological conditions. Moreover, preserve cells function after injury.
	↑	↓	↓↓↓	Oncogenic transformation (cancer).
Stress	↑	↑	↓	Transient reduction of mitochondrial function lead to increase ER-stress and UPR to promote cell survival.
	↑↑	↓	↑	Uncontrolled increase of ER-stress by the UPR, increase persistent inflammation.
Autophagy	↑	↑	↓	Promote cell survival and reduce cell damage.
	↓	↓	↑	Promote mitochondrial dysfunction/ inflammatory phenotype.
p53	↑	↑	↑[b]	Prevent damaged cell accumulation and restore the homeostasis in response to acute stress.
	↑↑	↓	↑↑↑	Decrease stem cell regenerative function and promote senescence in response to chronic stress
	↓	↓	↓↓↓	Oncogenic transformation (cancer).
TERT	↓	↓	↑	Cell death.
	↑	↑	↓	Preserve postmitotic and mitotic cell function after damage/injury.

***[a]** Apoptosis transiently decrease**; [b]**Apoptosis transiently increase.*

mTOR, mammalian target of rapamycin; I/IGF, insulin/insulin-like growth factor 1; AMPK, AMP-activated protein kinase; SIRT1, Sirtuin 1; ER stress, endoplasmic reticulum stress; UPR, unfolded protein response; TERT, telomerase reverse transcriptas.