Association Between Aging, Apoptosis And Related Dysregulations

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Abstract — *Aging or Apoptosis research is a rapidly developing area, but the role of apoptosis is still ambiguous and controversial. Aging is more closely related with a progressive deterioration of tissues and organs. While, many hypotheses have been proposed to explain the aging process, the exact mechanism involved is not chiseled. One of the most common characteristic to aging tissues is cell loss and modification in cell death signaling cascades could contribute to cell loss and underlie a number of age-related organ-specific cellular degenerations. Current review paper will focuses on apoptosis and hashes out how dysregulation of the apoptotic signaling cascade could be involved in aging and age-related pathologies. Furthermore, Apoptosis, or programmed cell death, is a biological process that begins during development and continues throughout adulthood. There is evidence that advanced age is associated with dysregulation of apoptosis. Our paper will discourse studies that show age-related changes in the proteins that regulate apoptosis and demonstrate that aging enhances apoptosis and increases the susceptibility of several different cell types to apoptosis. Modification in apoptotic signaling may help to explain the age-associated decrease in immunity, cardiac function in old age and also in the enhancement of neurodegenerative diseases in aging mankind.*

Index Terms— Apoptosis, Aging, Mitochondria, caspase

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1 INTRODUCTION

THE apoptosis is not a new word in the sciences to know about it but to understand it we have to look for recent research. In 1951, Gluckmann first described the apoptotic process. In 1972, Kerr, Wyllie, and Currie coined the term apoptosis to describe a form of cell death with morphological characteristics that are distinct from necrosis [1]. Necrosis is a passive form of cell death that results from acute cellular injury, which causes cells to swell and lyse while apoptosis is an active process in which cells die by design and apoptotic bodies are removed without inflammation. These findings were largely ignored in the early 1980s, but since 1987 the number of papers in this field has been growing rapidly. Researchers became interested in apoptosis after it was demonstrated in the nematode Caenorhabditis elegans, followed by the identification of homologous death genes in other organisms [2]. For example, CED-9, CED-4, and CED-3 are homologous to the mammalian gene products for Bcl-2, Apaf-1, and caspase-9, respectively [1],[3][4].

Apoptosis can be divided into three nondistinct phases: an induction phase, an effector phase, and a degradation phase. The first phase, "induction phase" depends on death-inducing signals to stimulate proapoptotic signal transduction cascades. Some of these death-inducing signals include reactive oxygen and nitrogen intermediates, TNF-α, ceramide, veractivation of Ca2+ pathways, and Bcl-2 family proteins such as Bax and Bad [5]-[8]. In second phase, "the effector phase", the cell becomes committed to die by the action of a key regulator, that is, death domain activation on the cell surface, nuclear activators (such as p53), endoplasmic reticulum pathways, or activation of mitochondrial-induced pathways (release of cytochrome c or apoptosis-inducing factors). In third phase, "the degradation phase" involves both cytoplasmic and nuclear events. In the cytoplasm, a complex cascade of protein cleaving enzymes called caspases (cysteine proteases) becomes activated. In the nucleus, the nuclear envelope breaks down; endonucleases are activated, causing DNA fragmentation; and the chromatin condenses. Finally, the cell is fragmented into apoptotic bodies and phagocytized by surrounding cells or macrophages [1],[9].

One classic case of the role of apoptosis in development is the elimination of tissues, transitory organs, and phylogenetic evidence. For example, the pronephros and mesonephros are eliminated by apoptosis in higher vertebrates. Anuran tails and gills undergo apoptosis as tadpoles change into frogs. Moreover, the roundworm C. elegans eliminates exactly 131 of its initial 1090 cells as it changes into its adult form [10]. Another classic example of programmed cell death is tissue remodeling. As

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vertebrate limb buds develop, for example, in chick, duck, and humans, webbing between digits in the hind limbs is removed by apoptosis. This indicates that the ectoderm sends signals to initiate programmed cell death [10]. In these cases, aging is under strict genetic control and therefore is truly programmed cell death. However, senescent aging—such as found in recent studies— may largely be due to "wear and tear" mechanisms [11]. It is also possible that a programmed mechanism becomes activated with aging that triggers cell loss; future investigations will have to distinguish between these two possibilities [11].

2   APOPTOSIS

Cells dying by apoptosis fragment into membrane-bound apoptotic bodies that are readily phagocytized and digested by macrophages or by neighboring cells without generating an inflammatory response. Apoptotic cells classically exhibit nuclear condensation and fragmentation, DNA laddering (200 bp fragments), blebbing or rounding of the cell, and the externalization of phosphatidylserine [12]-[15]. If the decision is cell death, the end point of the signaling cascade is the activation of an evolutionarily conserved family of cysteine proteases called caspases [15],[16]. Caspases are present in cells as inactive zymogens and are triggered by proteolytic processing. Once activated, caspases are involved in the downstream processing of the substrates required to dismantle the cell, including protein kinases, signal transduction proteins, cytoskeletal and nuclear matrix proteins, chromatin modifying enzymes PARP (poly (ADP ribose) polymerase), DNA repair proteins, and the inhibitory subunits of certain endonucleases [12].

2.1   Activating apoptosis

Two major pathways have been described that regulate apoptosis: the extrinsic pathway and the intrinsic pathway [13]. During extrinsic cell death signaling, the binding of extracellular ligands to receptors located in the plasma membrane induces trimerization of proteins known as death receptors, namely CD95 (Fas/Apo-1), TNFR1 and 2.

Ligands that activate these receptors are structurally related molecules that bind to the TNF superfamily. The CD95 ligand (CD95L) binds to CD95, TNF binds to TNFR1, etc. Activation of these receptors induces trimerization and the recruitment of cytoplasmic adaptor molecules including FADD (Fas associated death domain containing protein), TRADD (TNFR-associated death domain), or RIP (receptor-interacting protein). Recruitment of these adaptor proteins results in the recruitment of pro-caspase-8 to a protein complex known as the Death-Inducing Signaling Complex (DISC). Caspase-8 is activated within this complex and may induce cell death through the cleavage of caspase-3/-7 or through the cleavage of Bid and the subsequent activation of the intrinsic signaling pathway (Figure 1a) [12],[16]-[18].

The Granzyme B-perforin pathway is a mechanism by which cytotoxic T cells and Natural Killer (NK) cells eliminate target. Upon target cell engagement, immune cells release granules containing Granzyme B and the pore-forming protein, perforin. Once in the cytoplasm, Granzyme B will induce cell death primarily through cleavage of the protein Bid and the subsequent activation of the intrinsic apoptotic pathway (Figure 1b). Granzyme B will also target other intracellular substrates to induce apoptosis, including caspases-10 and caspase-3, and several caspase substrates such as the inhibitor of caspase-activated deoxyribonuclease (ICAD), reviewed in [19],[20].

Cell death signals originating from within the cell signal through the intrinsic or mitochondrial-mediated pathway. In mammals, internal death stimuli directly or indirectly change mitochondrial membrane permeability causing the release of cytochrome c from the mitochondrion into the cytosol where it binds to the adaptor molecule, Apaf-1 (apoptotic protease activating factor-1) [21]-[23]. This binding triggers the formation of a protein complex known as the apoptosome, which contains Apaf-1 molecules, cytochrome c, and procaspase-9 molecules [17,[24]. The procaspase-9 molecules bound to the apoptosome are activated and subsequently activate downstream caspases such as caspase-3 (Figure 2) [16],[17].

2.2   Regulating apoptosis

Most cells constitutively express caspase zymogens at levels sufficient to bring about cell death and the key to cell fate depends on the levels of active caspases in the cell. The triggering of death pathways depends not only on the activation of effector molecules but also on the mechanisms that counteract the pro-death signals. In mammals, the molecules that counteract the pro-death signals are the Bcl-2 and IAP families of proteins.

2.2.1   Bcl-2 proteins

Proteins of the Bcl-2 family are critical regulators of apoptosis, whose primary functions include governing mitochondrial-dependent cell death [21],[22]. Bcl-2 proteins directly regulate the permeability of mitochondrial membranes by either permitting or inhibiting the release of apoptogenic proteins such as cytochrome c. The Bcl-2 protein family has at least 20 members that have been identified in mammals [25],[26], and that can be

broken down into three groups; the pro-apoptotic multi-domain proteins (Bax, Bak, Bok); the pro-apoptotic BH3-only proteins (Bad, Noxa, Puma, and
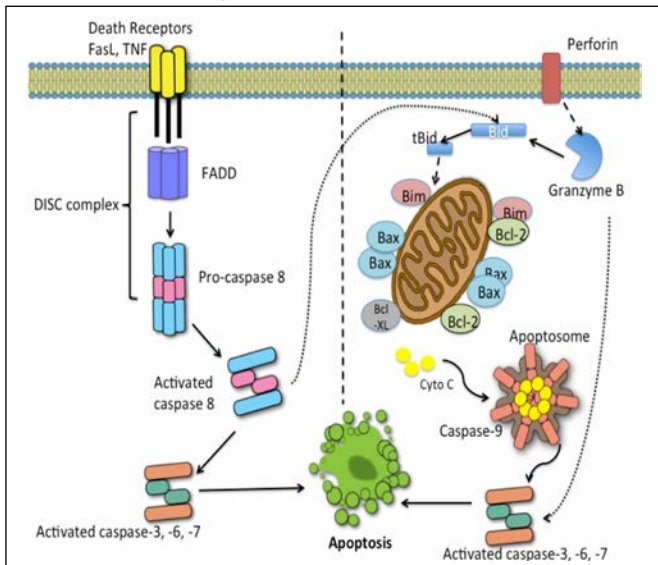

Figure 1: Simplified extrinsic apoptotic cell signaling.

(a) Death-Receptor signaling (left) is triggered by members of the death receptor superfamily (FasL, TNF). Binding of FasL to Fas induces receptor clustering and formation of the Death-Inducing Signaling Complex (DISC). This complex recruits and activates caspase-8. Active caspase-8 can induce apoptosis through direct cleavage of caspase -3, -7 or through the cleavage of Bid and subsequent triggering of the mitochondrial-mediated pathway.

(b) Classical Granzyme B/perforin-mediated pathway. Granzyme B delivery into the cytoplasm is facilitated by the pore-forming protein perforin. Once in the cytoplasm, GrB initiates apoptosis through the cleavage of Bid, which in turns triggers cytochrome c release, apoptosome formation, and caspase -3, -7 activation. Granzyme B can also bypass the mitochondrial-mediated pathway and initiate caspase activation directly and/or may cleave caspase substrates.

Bim), and the anti-apoptotic multi-domain protein proteins that include Bcl-2, Bcl- XL and Bcl-W. [27].

2.2.2   Inhibitors of apoptosis proteins (IAPs)

Given the irreversible nature of most proteolytic reactions, the most critical checkpoints for cell death regulation occur at the level of caspase activation. IAPs are a conserved family of caspase suppressors [28],[29]. The characteristic structural motif of all IAP family members is the presence of one to three Cys/His regions known as baculovirus IAP repeats, (BIRs) [30],[31]. BIR motifs mediate interactions with multiple death proteins, including the caspases. Many IAP proteins also contain a C-terminal Zinc RING finger. RING domains can act as E3 ubiquitin ligases and catalyze the transfer of ubiquitin to target proteins, including themselves, for ubiquitination. Five human IAPs have been characterized: NAIP1, XIAP, c-IAP1, c-IAP2, and survivin [28],[32]. In

general, IAPs suppress apoptosis by negatively regulating the activity of caspases [33],[34]. In mammals, caspase-9 is primarily inhibited by XIAP while caspase-3 and -7 are inhibited by XIAP and to a lesser extent c-IAP1, c-IAP2, and NAIP [34],[35].

2.2.3   Smac/Diablo and Omi/HtrA2

These two proteins negatively regulate XIAP and therefore regulate caspase activation. Both proteins contain an IAP binding motif through which they bind to IAPs and release IAP bound caspases. Smac/Diablo and Omi/HtrA2 are located in the
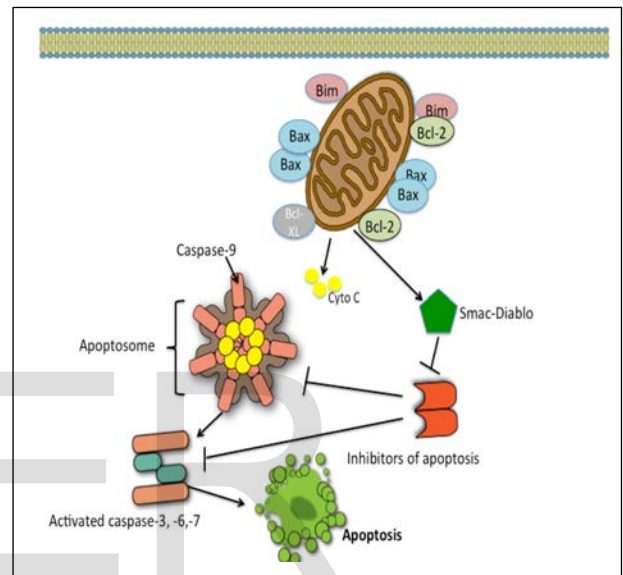

Figure 2: Schematic of the intrinsic pathway (mitochondrial-mediated).

The intrinsic apoptotic pathway is initiated by stress signals that alter mitochondrial membrane permeability and induce the release of apoptogenic factors such as cytochrome c and Smac/DIABLO. Mitochondrial membrane permeabilization is regulated by a balance of the apposing actions of pro-apoptotic and anti-apoptotic Bcl-2 family members (Bcl-2, Bcl-$_{XL}$, Bim, Bax, Bak, etc). The release of cytochrome c triggers the formation of the apoptosome; a protein complex containing caspase-9, APAF-1 and cytochrome c. Activated caspase-9 will activate caspase-3, -7 to induce apoptosis.

intermembrane space of mitochondria and are released into the cytosol during apoptosis, where they bind to IAPs releasing them from their caspases and thereby enabling the activation of caspases [36],[37].

2.3   Apoptosis and aging

The prevalence of apoptosis has been shown to increase with age in many different cell types, reviewed in [38]. To identify the molecular events associated with aging, a number of investigators have examined genome-wide changes associated with aging in various human and animal tissues [39]-[45]. Collectively, these studies have suggested that aging

results in a differential gene expression pattern indicative of altered apoptotic signals and stress responses, as well as changes to metabolic and biosynthetic signaling. In support of the genetic data, several in vitro and in vivo studies have provided strong evidence that changes in age-dependent apoptosis can be directly linked to changes in caspase expression and caspase activity. For example, age-dependent alterations in caspase activity have been observed for multiple caspases in rodent models of aging, including an increase in caspase-3, -6, -7, -9, -2 activity in the spleen, lung, and liver of old (24-26 months) compared to young (6 months) or middle aged (12-14 months) Fisher 344 rats [46]. Similarly, increased caspase-3 activity was reported in the hippocampus of aged (22 months) compared to young (4 months) rats [47]. In addition to changes in caspase activity, other markers indicative apoptotic activity have been detected in aging rats including elevated levels of cytoplasmic cytochrome c, and poly(ADP-ribose) (PARP) cleavage and terminal dUTP nick end staining [48],[49. Collectively, these investigations provide an association between increased apoptotic activity and the normal aging process in different tissues in aging animals.

2.4 Mitochondria, apoptosis and aging

The concept that mitochondria play a key role in the aging process is not new. Harman et al. [50] proposed the free radical theory of aging where oxygen free radicals are the cause of cumulative cellular damage that eventually results in aging and age-related pathologies in humans and animals. This theory was later refined to include mitochondria as key players in this aging process as they are the major source of free radicals in animal cells [51]. Linnane et al. [52] proposed the mitochondrial theory of aging where the accumulation of somatic mutations in mitochondrial DNA is a major contributor to the aging process. Here the progressive accumulation of mutations in mtDNA over an individual's lifetime leads to a decline in the bioenergetic function of mitochondria, dysfunction of the respiratory chain, increased production of reactive oxygen species (ROS) and the subsequent accumulation of more mutations in mtDNA.

In addition to being key mediators in the initiation and regulation of intrinsic apoptotic signaling in animal cells, mitochondria are also the major energy producers of the cell. Moreover, they are both the major source of reactive oxygen species (ROS) and free radicals under normal physiologic conditions, and are the major target for ROS and free radical damage within the cell. Studies from human and animal models have shown a wide spectrum of alterations in mitochondrial function and mtDNA

during the normal aging process that include; a decline in mitochondrial respiratory function, an increase in ROS production, an increase in the resulting damage to cellular constituents resulting from increased ROS production, enhanced mutations to mtDNA, and enhanced apoptosis, reviewed in [53].

Bioenergetic studies in human and animal models indicate that the respiratory functions of mitochondria decline with age in post-mitotic tissues. This is supported by studies reporting cytochrome c oxidase negative cardiomyocytes and muscle fibres in heart, limb muscles and diaphragm of elderly patients [54]-[56]. Further support is provided from observations that electron transport activities decline with age in the brain, skeletal muscle and liver of normal aging human subjects [57]-[59] and experimental animals [60],[61]. The above changes can be accompanied by a decline in ATP synthesis and increased ROS production in various human tissues [62]. Under normal physiologic conditions, ROS and free radicals are generated and maintained at a steady-state by mitochondria in animal tissues [63],[64] and the rates of ROS production from mitochondria are reported to increase with age in mammalian cell lines and tissues [65]-67]. Importantly, the oxidative damage associated with increased ROS production has been associated with damage to cellular constituents including lipids, proteins, and nucleic acids [68].

Oxidative damage can cause modifications to both nuclear and mtDNA and many of these modifications have been shown to increase with age [68]-[70]. MtDNA is particularly susceptible to oxidative damage because of its close proximity to sites of ROS production, lack of protective histones and limited capacity for DNA repair, reviewed in [53]. The age-associated increase in mtDNA mutations has been associated with reduced life span, and premature onset of aging-related phenotypes including alopecia, kyphosis, osteoporosis, anemia, and reduced fertility [71]. Furthermore, oxidative damage to mtDNA is reported to be major cause of instability and mutations in mitochondria that are associated with respiratory dysfunction, an increase in ROS production and a general decline in the efficiency of energy metabolism [52],[72]. In aging cells and tissues, these factors have been proposed to influence susceptibility to apoptosis and promotion of the aging process.

Lastly, phospholipids of mitochondrial membranes are extremely sensitive to oxidation. Alterations to these phospholipids can impact mitochondrial inner membrane barrier properties, maintenance of mitochondrial membrane permeability (MMP) and mitochondrial calcium buffering capacity [73]-[75]. Factors that affect (MMP), the regulation of pro- and anti-apoptotic Bcl-2

proteins, and the release of cytochrome c into the cytosol will lead to the activation of caspase-9-induced apoptosis. Studies in aging mice have shown that mitochondria from brain, liver and lymphocytes of older animals exhibit increased MMP activation. Similarly, the threshold for calcium-induced MMP has been shown to decrease with age in the lymphocytes, brain and liver in older mice, which in turn was found to lower the threshold for the release of apoptogenic factors such as cytochrome c into the cytosol and the subsequent induction of apoptosis [76]. In aging humans and animals, increased oxidant production is also associated with elevated levels of calcium in the cardiomyocytes [77] and elevated levels of cytosolic cytochrome c have been observed in the heart cells of aged (24 months) compared to young (6 months) male Fisher 344 rats [78].

2.5 Aging and Age-related Pathologies

2.5.1 Neurodegenerative disorders

Apoptosis plays a pivotal role in the progression of a variety of neurodegenerative diseases. Despite the many causes of such diseases, caspase activity has been reported across a broad spectrum of neurodegenerative diseases; however, the trigger for aberrant caspase activation is not well understood. The first evidence of a role for caspases in neurodegenerative diseases came from experiments in mouse models of ALS, where prolonged caspase activation was detected in ALS-transgenic mice. In this model, caspase-1 and caspase-3 transcripts were up-regulated as these mice aged [79]-[81].

Alzheimer's disease (AD) is the most common form of dementia and is characterized by progressive impairment of cognitive function. Histologically, AD is associated with senile plaques containing amyloid-β peptide (Aβ) and neurofibrillary tangles composed of hyperphosphorylated tau proteins and paired helical fragments [82]. Several studies have reported a link between multiple caspases and AD, reviewed in [83]. Both in vitro and in vivo studies have reported elevated expression and activation of caspases in animal models of AD [84]-[88]. Moreover, elevated levels of caspases are found in the brain of severe definitive cases of AD [88]-[94]. Pompl et al. [95] showed elevated expression of caspases-1, -2, -3, -5, -6, -7, -8 and -9 in the brain of patients with AD compared with controls. Pyramidal neurons from vulnerable regions of the brain are reported to show an increase in activated caspase-3 and caspase-6 [94], and synaptosomes from AD brain frontal cortices have shown an enrichment in caspase-9 compared with non-demented controls [91],[93]. Strong evidence linking caspase activity to the development of AD are provided by studies showing that amyloid

precursor protein (APP) can be cleaved by caspase-3, -6, -7 and -8 [84],[93] and amyloid plaques are enriched in caspase-cleaved APP [96]. Further evidence is provided from studies in caspase-2 and caspase-12 knockout mice that demonstrate neurons resistant to Aβ [87],[97]. In addition to APP, caspases will also cleave the protein tau, thereby enhancing tau filament polymerization in vitro [98],[99]. Taken together, these data demonstrate that caspases are involved in the pathogenesis of AD but that further studies are required to refine the roles played by each specific caspase. It should also be noted there is evidence linking caspase activity to the development of AD, Parkinson's and dementia resulting from human immunodeficiency virus [100],[101].

Lastly, there is growing evidence for a link between the Bcl-2 proteins and the development of AD. Lu et al. [102] showed Bcl-2 and Bax are found to co-localize in the frontal cortex of patients with AD. Further support for the involvement of Bcl-2 proteins in AD pathogenesis comes from in vitro studies showing that Aβ induces an up-regulation of pro-apoptotic molecules such as Bax and a down-regulation of anti-apoptotic molecules such as Bcl-2, Bcl-XL, and Bcl-W [103]. One additional pro-apoptotic Bcl-2 family member, Bim (Bcl-2-interacting mediator of cell death) has been linked to AD. Bim is induced in both corticol and hippocampal neuronal cultures after Aβ exposures and its induction are essential for the neurotoxic effects of Aβ [104]. In conclusion, there is strong evidence to provide support for the hypothesis that apoptosis plays an important role in the neuronal loss observed in dementia and Alzheimer's disease.

Caspase activity has also been linked to the development of Huntington's disease. Huntington's disease is an autosomal dominant disorder in which specific cell death occurs in the neostriatum and cortex [48]. Two features commonly associated with this neurodegenerative disease are mutations associated with the protein Huntingtin, and neuronal dysfunction associated with the down-regulation of neurotransmitter receptors [105],[106]. One of the earliest events in the progression of Huntington's is the up-regulation of caspase-1 gene expression [106]. As the disease progresses, caspase-3 transcription is up-regulated and elevated levels of caspase-3 activity are detected [107]. Moreover, caspase-8 and caspase-9 activation, as well as the release of cytochrome c have also been demonstrated in Huntington's disease [108],[109]. While the direct cause of aberrant caspase activity is not fully understood in many neurologic disorders, there is strong evidence directly linking caspase activity to the progression of Huntington's disease. Here, Huntingtin, a protein believed to be central to the development of Huntington's disease, is a substrate for both caspase-1 and caspase-3

[110],[111]. As the disease progresses, increased caspase-mediated cleavage of Huntingtin increases the levels of Huntingtin fragments and depletes the levels of wild-type Huntingtin [106]. Both are thought to play a role in Huntington's disease progression. Lastly, caspase activity is believed to play a role in the development of neuronal dysfunction as the inhibition of caspase activity also inhibits the down-regulation of neurotransmitter receptors in a mouse model of Huntington's disease [106]. Transgenic mice have been used as a tool for evaluating the efficacy of caspase inhibitors in animal models of Huntington's disease [100],[106],[107].

2.5.2   The aging immune system

The deterioration of the immune system is believed to contribute to morbidity and mortality in aging humans. Certainly, older animals are more susceptible to microbial infections and demonstrate an increased prevalence of specific cancers and certain autoimmune diseases with advancing aging. Aging is associated with thymic involution, lymphopenia, and a progressive deterioration in T cell function, reviewed in [112]. This decline in T cell function is believed to play a role in age-enhanced susceptibility to infection, autoimmunity and cancer [113]-[115]. Apoptosis is vital in controlling T cell number, deleting self-reactive cells and maintaining immune surveillance during development and tissue homeostasis. Age-dependent increases in apoptotic activity and caspase activity have been reported in both human and murine T cells and B cells, reviewed in [116]. Lacelle et al. [117] used quantitative RT-PCR to screen the PBMCs from human subjects ranging from 2-102 yrs of age where they observed an increase in caspase-1 and caspase-3 mRNA levels in old (70-89 years) and extremely old (>90 years) humans compared to those in younger age groups. In this same study, old individuals were also reported to express higher levels of caspase-8 mRNA. Similarly, Aggrawal et al. [118] reported increased expression of caspase-8 and caspase-3 protein has been reported in T lymphocytes of aging human subjects. Moreover, CD4+ and CD8+ T cells from older human subjects are reported to display increased expression of TNFR1 and TRADD, and increased caspase-8 and caspase-3 activity upon stimulation with TNF-α. Given that TNF-α levels are reported to increase with age, this suggests that T cells from older individuals may be more sensitive to apoptotic stimuli [113],[119]. In addition to increased caspase activity, lymphocytes from older subjects have been found to express higher levels of Fas and Fas ligand, increased expression of FADD, increased expression of the pro-apoptotic protein Bax and decreased expression of anti-apoptotic protein Bcl-XL [113],[120]. While these data

suggest that apoptotic activity increases with age, it is unclear if the increased apoptotic activity is the result of altered cell signaling during the aging process or an increased sensitivity to apoptosis. Either scenario in lymphocytes may help to explain a general decline in immunity with aging humans.

In addition to the observed increase in peripheral lymphocyte apoptosis, hypocellularity of primary lymphoid organs is a distinctive characteristic of aging humans. The thymus is known to atrophy with progressive aging resulting in a significant loss in its capability to generate new T cells for export into the peripheral T cell pool [121]. There is a clear increase in the number of apoptotic cells in the thymic cortex with aging. Histologically, [122] showed an increase in the apoptotic index of the thymic cortex in 7-month old mice compared to 1-month old mice. The noted increase in apoptotic activity was accompanied by a marked decline in proliferation suggesting that apoptosis may account for the reduction of thymic cortical cellularity during aging. Support for this hypothesis is provided by other studies showing an age-associated increase in thymocyte loss associated with enhanced expression of the pro-apoptotic genes Bax and p53, and with increased the cleavage of poly (ADP-ribose) polymerase (PARP) and increased caspase-3 activation [49],[123]. Collectively, these alterations in apoptosis-related components create a scenario where the balance between survival and death is altered in aged lymphocytes and lymphoid organs and may provide a mechanism for reduced immunity in aging animals.

2.5.3   Cardiovascular system

There is increasing evidence of a relationship between apoptosis and cardiovascular disease, particularly for the heart diseases common to the elderly populations, including ischemic heart disease and congestive heart failure. The aging process in the heart is characterized by a significant loss of cardiac myocytes with the base-line level of apoptosis higher in older compared to younger animals [124]. This age-associated elevation in apoptotic activity is clinically relevant, as the adult heart cannot repair damaged tissue due to the fact that mature cardiomyocytes are terminally differentiated and therefore unable to divide. The loss of cardiomyocytes with age may ultimately lead to impaired cardiac function with [125]. Evidence of age-associated elevations in cardiomyocyte apoptosis has been noted across different several different species (humans, mouse, rabbit, dog, sheep and pig) and is observed in different experimental models including myocardial infarction (MI) [126]-[131]. Kajstura et al. [124] estimated the initial ventricular cardiomyocyte population to decline by as much as 30% as the heart

ages. Moreover, increased levels of cardiomyocyte apoptosis are detected following ischemic attacks suggesting that apoptosis in the aging heart is a contributing factor for the elevated MI-related morbidities and mortalities observed in elderly patients [131]-[133].

The underlying mechanisms for apoptosis in the aging heart are not fully understood; however, there is strong evidence for alterations in mitochondrial oxidative stress in aging cardiomyocytes, including elevated levels of diastolic Ca2+ and elevated levels of cytochrome c [76]-[78],[124]. Interestingly, while several studies have shown progressive changes in intracellular Ca2+ levels and cytoplasmic cytochrome c levels, studies that have examined other markers of mitochondrial-mediated apoptosis, namely the Bcl-2 family of proteins, have shown interesting but occasionally conflicting results. Phaneuf et al. [78] have shown an increase in cytosolic cytochrome c in 16-24 month animals compared to 6-month animals, accompanied by an age-dependent decrease in mitochondrial Bcl-2 levels but no alteration in mitochondrial Bax levels. In contrast, studies in spontaneously hypertensive rats (SHR) have shown significantly elevated ratios of Bax/Bcl-2 that correlate with an increase in apoptosis that begins at 4 weeks and is maintained at high levels throughout aging [134]. Similarly, in ischemia-reperfusion models of cardiac injury, Liu et al. [135] reported significantly increased levels of Bax in aged compared to young hearts. It is interesting to note that transcriptional analysis of apoptotic genes in the aging heart have shown that caloric restriction can down-regulate the expression of pro-apoptotic factors, such as Bax and caspase-9, as well as up-regulate the expression of pro-apoptotic genes such as IAPs and Bcl-2 [42]. Taken together, these studies suggest an age-dependent elevation in mitochondrial oxidative stress may lead to alterations to the relative amounts of pro- and anti-apoptotic proteins, which can be modified by caloric restriction. Whether this imbalance is involved in altered apoptotic signaling that directly impacts the aging process or whether these changes simply represent an increased sensitivity to apoptosis in aging tissues requires further elucidation. Strategies aimed at promoting cardiomyocyte survival and proliferation will provide new therapies to reduce the susceptibility of aging hearts to ischemic injury and end-stage heart failure.

It is also worth noting that in addition to age-related cell loss, apoptosis has been linked to other age-related vascular diseases, including atherosclerosis. Atherosclerosis is a lipid-driven inflammatory disease responsible for the majority of heart attacks, and lower limb loss [136]-[138]. T cells and macrophages are abundant in atherosclerotic lesions where they are known to participate in the inflammatory response and the induction of apoptotic cell death. Here, apoptosis has been observed during the early stages of lesion development where smooth muscle cell apoptosis may be a beneficial way to reduce intimal hyperplasia [139]. However, in more advanced lesions, apoptosis may decrease plaque stability through decreased cellularity and extracellular matrix degradation [19],[140].

2.6 Other cells and organ systems

The prevalence of apoptosis has been shown increase with age in many other cell types and organ systems. Age-associated increases in chondrocytes have been observed on all articular surfaces of the knee joints of C57BL/6 mice and Wistar rats [141], suggesting that apoptosis plays a role in the maintenance and remodeling of mature articular cartilage. Granzyme B-induced apoptosis has been implicated in cartilage matrix destruction and is thought to be an important mediator in the pathogenesis of reactive arthritis [142],[143], as well as contribute to cartilage degeneration over time [19]. There is also strong evidence that the age-associated loss of skeletal muscle mass is related to apoptosis in both human and animal models, reviewed in [144]. Finally, age-associated increases have also been observed hepatocytes [145]-[148], β cells in pancreatic islets [149]-[151] and oocytes [149],[150].

It is important to note to that age-dependent alterations in apoptotic activity are not limited to increases in apoptotic activity as decreased caspase levels and activities have also been observed in aged tissues. For example, decreased levels of caspase-3, caspase-8 and caspase-9 have been reported in the colon mucosa of old (22-24 months) F344 rats compared to young (4-5 months) and middle aged (12-14 months) rats [152]. This observed decrease in caspase activity was accompanied by a decrease in PARP cleavage, as well as decreased levels of Bak, and increased levels of the anti-apoptotic protein Bcl-XL. Moreover, the observed decrease in caspase expression and activity was associated with lower numbers of apoptotic cells in colon mucosa in older rats. Similar results are reported in patients with acromegaly [153]. These alterations may help to explain the increased incidence of colon cancer in older individuals.

3 CONCLUSION

In summary, Aging is a natural and complex biological process and many studies have shown that aging is associated with altered cell signaling. Apoptosis is involved in aging and age-related disease, however, apoptosis serves to eliminate presumably dysfunctional cells and protect organism

from cancer. It has been well established that apoptosis plays a role in the aging processes as age-related alterations in apoptotic proteins have been observed in various cells type and a number of different organ systems. Although an increase in apoptotic cell death has been reported with aging in both human and animal models, it is often difficult to determine if it is altered apoptotic signaling or simply normal apoptotic activity over-time that is contributing to the observed age-related phenotypes. Though, apoptosis is, without question, critical for homeostasis, there is evidence that even normal apoptotic processes might, over-time lead to normal aging phenotypes or age-related pathologies, particularly in post-mitotic cells such, as neurons and cardiomyocytes.

Research of apoptosis and aging are ascertained by the same troubles that plague the studies of apoptosis in general, namely the differential specificity of the techniques used to detect apoptosis and the challenges in translating and linking these findings to a particular phenotype or clinical consequence. A closer understanding of how apoptosis relates to aging and age-related pathologies will be vital, if we are to better manage age-related diseases or develop strategies for intervening in the aging process. Future investigations will have to examine the molecular mechanisms behind aging interventions and their effects on the rate of apoptosis and relevant apoptotic pathways.

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