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Review Article

Role of Apoptosis in Sarcopenia

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Skeletal muscle atrophy and the loss of myofibers contribute to sarcopenia, a condition associated with normal aging. However, relatively little is known regarding the relevance of apoptosis to skeletal muscle homeostasis and the possible mechanisms involved, although evidence suggests that apoptosis may play a role during muscle aging. By age 80 it is estimated that humans generally lose 30%–40% of skeletal muscle fibers, particularly from muscles containing type II fibers such as the vastus lateralis muscle. Studies using rodents show that between a 20%–50% loss in muscle fibers occurs depending on the specific fiber type studied. Caspases (cysteine-dependent, aspartate-specific proteases) such as caspase-3 play an important role in mediating cell death in that many of the apoptotic signaling pathways, such as the mitochondrial-mediated, receptor-mediated, and sarcoplasmic-reticulum-mediated pathways, converge at caspase-3 in the caspase cascade. Studies show that with age the levels of several caspases are significantly increased. Therefore, the activation of these proteolytic caspases may be partly responsible for the initiation of muscle protein degradation, loss of muscle nuclei, which is associated with local atrophy, and finally into cell death of the myocyte.

APOPTOSIS is a highly regulated form of cell death that is characterized by specific morphological, biochemical, and molecular events (1–5). It is important to normal development of multicellular organisms and is involved in cell turnover and remodeling in healthy and diseased tissue (1–4). Accelerated apoptosis with normal aging has been reported in several mitotic tissues, such as liver and white blood cells, and may serve, respectively, to prevent age-associated tumorigenesis and to maintain overall control of immunocompetent cells (6,7). In contrast, apoptosis in postmitotic tissues, such as the brain, heart, and skeletal muscle, may result in diminished tissue function with normal aging (5,7–10), although this possibility has not been well investigated. Several stimuli exist, for example calcium, physiologically produced oxidants (hydrogen peroxide, peroxynitrite), and tumor necrosis factor alpha (TNF-α), which can initiate apoptotic signaling. Since aging is associated with increased mitochondrial oxidant production and oxidative damage (11–13), mitochondrial dysfunction may trigger the initial events of mitochondrial-mediated apoptosis via release of proapoptotic proteins into the cytosol (14,15). In addition, there is evidence that cytosolic Ca^{2+} levels increase with age (16,17), providing a favorable environment for the activation of the endoplasmic reticulum-mediated apoptotic pathway. Finally, increased levels and production of TNF-α by aged skeletal muscle (18) may act as a signal to activate death receptors on the cell surface membrane.

Apoptotic pathways can activate *c*ysteine-dependent, *aspar*tate-specific protea*ses* (caspases), which are endoproteases and integral to the final execution of cell death. Caspases normally exist in an inactivated state in the cytoplasm but can be activated by proteolytic cleavage and subsequent heterodimerization (19). Apoptotic ''effectors'' (i.e., caspase-3) carry out the actual proteolytic events that result in cellular breakdown. Once the proteolytic cascade is switched on, it eventually cleaves and activates procaspase-3 and further initiates the caspase cascade, which leads to the disassembly of the cell (20). The mitochondrial-mediated pathway has recently been implicated as a major regulatory center for apoptosis and operates through the activation of the upstream-located procaspase-9 (14,15,21). The mitochondrial-mediated pathway may operate in several different ways. It can release cytochrome-c from the mitochondria, forming an ''apoptosome'' (Apaf-1, adenosine triphosphate, cytochrome-c), which activates procaspase-9 into active cleaved caspase-9. Alternatively, it releases proapoptotic proteins, such as apoptosis-inducing factor (AIF) and Omi, which operate independently from caspases (22,23). Several other pathways require an alternate upstream activator(s) to initiate the caspase cascade (i.e., caspase-8, caspase-10, caspase-12; apoptotic ''initiators''). Receptor-mediated pathways can be activated by TNF-α binding to its death domains and induce apoptosis in an effector cell by the activation of procaspase-8, which cleaves and activates procaspase-3 to initiate the caspase cascade (20). Alternatively, endoplasmic reticulum stress could also contribute to apoptosis by releasing calcium into the cytosol and thereby activating m-calpain, procaspase-7, and procaspase-12 (24).

There is evidence indicating that apoptosis plays a key role in pathophysiological skeletal muscle cell loss. Skeletal muscle apoptosis has been documented to occur in muscular dystrophy (9,25), during chronic heart failure (26), skeletal muscle denervation (27), muscle unweighting or unloading (28), and during acute exercise (9,25). For example, Borisov and Carlson (27) found that denervating muscle for 2 and 4 months showed extensive nuclear fragmentation with the terminal (TdT)-mediated dUTP-biotin nick end labeling method (TUNEL). In contrast, the incidence of apoptosis in

skeletal muscle with age and its mechanisms have not been well investigated and require further research. It has been shown that muscle mass and fiber number decrease significantly with age (5,29–33). For example, an age-dependent increase in apoptosis of the striated muscle fibers of the rhabdosphincter led to a dramatic decrease in the number of striated muscle cells in humans (34). In a 5-week-old neonate, 87.6% of the rhabdosphincter consisted of striated muscle cells, while in a 91-year-old, only 34.2% of the rhabdosphincter consisted of striated muscle cells. We recently quantified apoptosis in the gastrocnemius muscle in 6-month-old and 24-month-old male Fischer 344 rats (35) and found a 50% increase in cytosolic mononucleosomes and oligonucleosomes in the 24-month-old animals compared with the 6-month-old animals. Apoptosis results in the activation of endonucleases, caspase-3-mediated activation of nucleases, which cleave double-stranded DNA between nucleosomes. Therefore, our study strongly suggests an increase in nuclei loss and/or apoptotic cell death in skeletal muscle (35) with age. Several scientists have implicated the mitochondria as a key player involved in sarcopenia. Cortopassi and others (5,36,37) suggested that mitochondrial dysfunction and oxidant stress to mitochondria could induce the mitochondrial permeability transition, the release of cytochrome-c, and subsequent initiation of apoptosis. Furthermore, Fitts and colleagues (38) showed increases in glycolysis and glycogen utilization during contractile activity in aged rats, suggesting an increase incidence in mitochondrial dysfunction with age.

Since skeletal muscle is a multinucleated cell, reported data on the occurrence of skeletal muscle apoptotic nuclei in human or animal disease models range between 0.03% and 2.1% (39). To enter into the discussion about the significance and relevance of apoptosis in skeletal muscle, it is important to ask the following questions: How long is an apoptotic nucleus detectable? Will it actually be lost? and What would be the relevance of this loss of nuclei to myocyte integrity and function? Recently, evidence has accumulated supporting a role for the modulation of myonuclear number during muscle remodeling in response to injury, aging, adaptation, and disease (40). It is suggested by Allen and colleagues (40) that muscle atrophy and disease are associated with the loss of myonuclei, possibly through apoptotic-like mechanisms. Indeed, in skeletal muscle of old rats there is strong evidence that specific muscle regions undergo atrophy, contain cytochrome-c oxidase negative fibers (indicative of mitochondrial dysfunction), have extensive MtDNA deletions, and have a significantly reduced number of nuclei (31). With age, this scenario could be responsible for a substantial loss of muscle mass and eventually loss in entire fibers affecting skeletal muscle function.

The mechanism(s) involved in the loss of nuclei remains unknown. Recently, we showed that a novel protein, AIF, is increased in skeletal muscle cytosol with age. Mitochondria can release AIF, and following AIF translocation to the nuclei, it causes DNA fragmentation (35,37,41). The AIF protein has recently been highlighted in a *Nature* article as a key programmed cell death pathway that may compensate for caspase-dependent apoptosis (41). Thus, myonuclear domain size could be dramatically reduced with age due to mitochondrial release of AIF, leading to the fragmentation of neighboring nuclei (41).

In summary, the role of apoptosis in age-related skeletal muscle nuclei and cell loss is unknown, and the apoptotic stimuli and signaling pathways that may be activated are unknown. We believe that calcium and hydrogen peroxide are the key signals to initiate cellular activation of apoptotic pathways, such as the mitochondrial and sarcoplasmic reticulum-mediated pathways. By the year 2030, the elderly population will grow from 13% to approximately 20%, and it is estimated that $130 billion will be imposed by physical frailty (42–46). In addition, frailty is a major predictor of poor outcomes such as hospitalization, nursing home placement, and death (42–46). Therefore, identifying the signal transduction pathways responsible for the age-related increase in apoptosis will permit the development of interventions that could prevent the loss of skeletal muscle myocytes and therefore attenuate sarcopenia.

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