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Apoptosis in skeletal muscle and its relevance to atrophy

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Abstract

Apoptosis is necessary for maintaining the integrity of proliferative tissues, such as epithelial cells of the gastrointestinal system. The role of apoptosis in post mitotic tissues, such as skeletal muscle, is less well defined. Apoptosis during muscle atrophy occurs in both myonuclei and other muscle cell types. Apoptosis of myonuclei likely contributes to the loss of muscle mass, but the mechanisms underlying this process are largely unknown. Caspase-dependent as well as -independent pathways have been implicated and the mode by which atrophy is induced likely determines the apoptotic mechanisms that are utilized. It remains to be determined whether a decrease in apoptosis will alleviate atrophy and distinct research strategies may be required for different causes of skeletal muscle loss.

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INTRODUCTION

Apoptosis, or programmed cell death, is an important process in multicellular organisms both during development, where it ensures the elimination of superfluous tissues,

and in adulthood, where it is critical for maintenance of tissue homeostasis. The early stage of apoptosis involves death-inducing signals, such as reactive oxygen and nitrogen species, ligands for the death receptors, imbalances in calcium regulation, and alterations in the composition and abundance of B-cell lymphoma (Bcl)-2 family proteins, such as Bax, Bad, Bcl-2, Bcl-xl (see^[1] for review). After this induction phase, nuclear activators, cell surface receptors, or mitochondrial pathways become activated in the commitment to cellular death followed by cytoplasmic and nuclear events. During apoptosis, protein-cleaving enzymes, i.e., caspases (cysteine-dependent aspartate-directed proteases), become activated in the cytosol and are responsible for proteolytic cleavage of a broad spectrum of cellular targets^[2]. Caspase-independent mechanisms also exist, such as the release of apoptosis-inducing factor (AIF) and endonuclease G (EndoG) from mitochondria (Figure 1), inducing large scale DNA fragmentation and apoptosis after translocation to the nucleus^[3-5]. In the nucleus, DNA fragmentation caused by activated endonucleases, chromatin condensation, and the breakdown of the nuclear envelope occurs and eventually the cell itself disintegrates into apoptotic bodies and is phagocytosed by surrounding cells or macrophages^[6]. Both extrinsic or intrinsic stimuli can be responsible for the induction of apoptosis, with some cross-talk between signaling pathways^[1,7,8].

ROLE OF APOPTOSIS

In highly proliferative tissues, such as the intestinal epithelium, apoptosis serves to maintain a constant number of cells and consistent tissue architecture, counterbalancing the rapid proliferation. Indeed, cell loss in the normal intestine can largely be explained by apoptosis^[9]. In addition, in the small intestine apoptosis serves to stabilize the stem cell population by removing excess, or perhaps compromised, stem cells^[10]. Decreased rates of apoptosis have been observed during tumor progression in colon carcinomas^[11] and the overall decrease in apoptosis in proliferative tissues may predispose the cells in those tissues to accumulate genetic changes characteristic of tumorigenesis.

In contrast, in post mitotic tissues such as skeletal muscle, the role of apoptosis is less clear. Also, with respect to cell death, skeletal muscle is a unique tissue because muscle cells, i.e., myofibers, are multinucleated. This aspect of skeletal muscle has led to the concept of the myonuclear domain, which is defined as the theoretical amount of cytoplasm supported by a single muscle fiber nucleus, which is called a myonucleus (protein/DNA)^[12].

Even though muscle size can vary considerably under different conditions, the myonuclear domain size remains relatively constant, implying a fairly strict regulation of myonuclear number (for review^[13]). Myonuclear number decreases in muscles undergoing atrophy in a variety of experimental conditions, such as spinal cord isolation and transection, microgravity, hind limb suspension, and chronic denervation^[14-20] and the process by which nuclei are eliminated from muscle fibers resembles apoptosis. Since destruction of the entire cell does not necessarily follow the elimination of a nucleus, as occurs with apoptosis in mononucleated cells, this process is called 'apoptotic nuclear death'. The mechanisms underlying apoptotic nuclear death in muscle are likely distinct from those involved in apoptosis in mononucleated cells.

The fact that apoptosis plays an important role in skeletal muscle atrophy can be deduced from the observation that it is increased in skeletal muscle in a number of pathological and under some physiological circumstances. Chronic heart failure, motor neuron disorders, skeletal muscle denervation, spinal cord injury, muscular dystrophy, and skeletal muscle atrophy due to hind limb suspension or immobilization are all associated with an increase in apoptosis in affected skeletal muscles, as measured by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) or by DNA fragmentation in gel electrophoresis^[16,21-26]. In addition, exercise was shown to increase apoptosis when assayed acutely after a bout of exercise^[24], but by contrast, exercise training for a period of 8 weeks decreased apoptosis^[27]. Interestingly, we and others have shown that exercise training attenuated the apoptosis induced by disuse (spinal cord injury or hind limb suspension)^[16,26]. This exercise-associated decrease in apoptosis under atrophy-inducing conditions may depend on the mode or intensity of exercise, since recently we found that gravity-independent resistance exercise did not decrease apoptosis during hind limb suspension^[28]. Therefore, apoptosis in skeletal muscle seems to be a highly regulated process that may serve distinct functions under different physiological and pathological conditions and a better understanding of pathways involved in the apoptotic response in muscle is warranted.

APOPTOTIC PATHWAYS IN SKELETAL MUSCLE

Caspases are thought to be the main enzymes involved in both the initiation (caspase-8, -9, -12) and execution of apoptosis (caspase-3, -6, -7) (Figure 1). Internal as well as external signals can activate caspases and this is under the control of the balance between pro- and anti-apoptotic proteins of the Bcl-2 family, heat shock proteins, and inhibitors of apoptosis proteins (IAPs)^[29]. However, apoptosis can ensue in the absence of caspase activation, whereas caspase activation does not always necessarily trigger cell death^[30]. Caspase-3 is often used as a surrogate for apoptosis, but in muscle, in particular, this may not be justified. We and others found that caspase-3 activity did not increase with atrophy induced by hind limb suspension^[31,32],

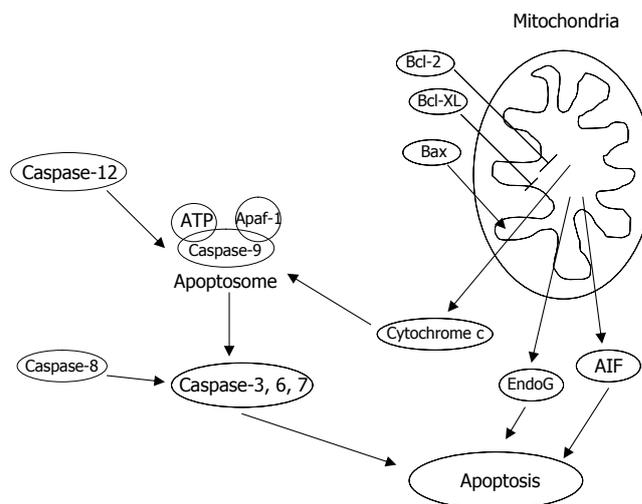


Figure 1 Pathways involved in apoptosis in skeletal muscle. Caspase-dependent and -independent pathways are likely involved in apoptotic nuclear loss during atrophy in skeletal muscle. Caspase-3 activation and subsequent apoptosis occurs through the release of cytochrome-c from mitochondria in response to changes in the ratio of pro- and anti-apoptotic members of the Bcl-2 family of proteins, or through the activation of initiator caspases, such as caspase-8 or -12. Caspase-independent pathways such as the release of EndoG and/or AIF may be more important in skeletal muscle as they directly induce DNA fragmentation.

even though apoptosis was increased concurrently, indicating that other pathways, besides caspase-3 activation, may be involved. In contrast, denervation-induced atrophy was associated with an increase in caspase-3 activity^[33] as well as caspase-8^[34], indicating that the activation of caspases may serve a different role in distinct models of muscle atrophy or that the mode by which atrophy occurs determines the involvement of different pathways. Indeed, caspases have been found to play non-apoptotic roles in pathways such as the inflammatory response, immune cell proliferation and differentiation of various cell types (e.g. skeletal muscle)^[35]. In skeletal muscle, caspase-3 was found to be involved in protein degradation, in particular of filamentous actin^[36], and it contributed to muscle weakness in response to endotoxin^[37]. Similarly, in cardiac muscle caspase-3 was shown to be involved in post-ischemic contractile dysfunction (cardiac stunning), apparently independent of apoptosis^[38]. Therefore, classical apoptotic pathways, as observed in mononucleated cells, may not be as important in muscle and other molecules may take on the role of apoptosis inducers.

In this light, we have investigated the role of EndoG during skeletal muscle atrophy^[31,39]. EndoG is a protein released from mitochondria upon pro-apoptotic stimulation and is capable of inducing DNA fragmentation independent of caspase activation^[40-42] (Figure 1). We found that EndoG co-localization with nuclei was increased in muscles atrophied in response to both hind limb suspension and age^[31,39] and that EndoG protein was increased in muscles of aged rats undergoing disuse-induced atrophy^[31]. Moreover, EndoG translocation was very specific for myonuclei and did not seem to be involved in the apoptosis of interstitial cells, which also occurs during muscle atrophy^[39]. In contrast, caspase-3 activation was observed more in interstitial cells and therefore different cell types within

the same tissue may be undergoing apoptosis through different mechanisms. Another protein released from mitochondria upon pro-apoptotic stimulation and capable of inducing apoptosis independent of caspases is apoptosis inducing factor (AIF)^[43]. Siu and Alway^[33] showed that AIF release was elevated in denervated muscle, in concert with cytochrome-c, Smac/DIABLO and a subsequent upregulation of caspase-9 and -3. Therefore, the atrophy-inducing stimulus, the time point after onset of disuse, or the different cell types undergoing apoptosis may be important in the activation of distinct apoptotic pathways. Finding interventions to counteract the increase of apoptosis with muscle atrophy will be challenging, considering the many different pathways used to induce apoptosis in skeletal muscle.

INHIBITION OF APOPTOSIS

The question arises whether inhibiting apoptosis in skeletal muscle will also decrease atrophy induced by disuse or due to aging. Recently, Siu and Alway^[44] showed that inhibition of apoptosis somewhat attenuated muscle atrophy induced by denervation in a Bax knock out mouse model. Interestingly, DNA fragmentation after denervation was much lower in the knock out mice compared to the wild type and caspase activation was also lower. However, mitochondrial AIF release was not decreased, possibly implying that caspase-independent mechanisms were not affected. In this study, the effect on different cell types was not investigated, but it is plausible that the minimal effect on muscle mass could be due to the fact that myonuclear apoptotic loss was not affected by the Bax-/- phenotype. Therefore, it remains to be determined whether inhibition of myonuclear apoptosis will decrease muscle atrophy.

CONCLUSION

Recently a number of studies have implicated an important role for apoptosis in the development of skeletal muscle atrophy. It is likely that atrophy induced by different conditions, such as denervation, microgravity, aging, cachexia, and spinal cord injury, initiates different apoptotic signals and indeed the role of apoptosis may be distinct among the conditions. It will be important to investigate, at the cellular level, which signals are responsible for the loss of myonuclei, interstitial cells, or stem cells in skeletal muscle in order to develop strategies to decrease apoptosis and atrophy.

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