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Review

Sensitivity of skeletal muscle to pro-apoptotic factors

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Abstract

In mononuclear cells, apoptosis leads to DNA fragmentation and cell destruction, regardless of the activated pathway. As regards multinuclear cells, e.g. skeletal muscle fibers, apoptosis rarely induces the death of the entire cell, and it generally affects single nuclei. This process, referred to as nuclear apoptosis, has a negative effect on the expression of genes in the myonuclear domain. Apoptosis may be initiated in muscle cells by external stimuli which activate cell membrane death receptors as well as by internal stimuli which stimulate the mitochondrial release of pro-apoptotic proteins. Reactive oxygen species also play an important role in the initiation of apoptosis. In muscle cells, ROS are produced in response to extracellular reactions or by cell mitochondria. It is, therefore, believed that mitochondria play a central role in apoptosis within skeletal muscle. Skeletal muscles have a well-developed system that protects them against oxidative damage. Myogenic stem cells are an integral part of multinucleated myofibers, and they are critically important for the maintenance of normal muscle mass, muscle growth, regeneration and hypertrophy. The latest research results indicate that myogenic cells are more sensitive to oxidative stress and pro-apoptotic factors than well-differentiated cells, such as myotubes. The complex structure and activity of skeletal muscle prompted research into the role of apoptosis and its intensity under various physiological and pathological conditions. This review summarizes the results of research investigating control mechanisms and the apoptosis process in skeletal muscle fibers, and indicates unresearched areas where further work is required.

Key words: apoptosis, skeletal muscle, satellite cells, regeneration

Introduction

Apoptosis is a well-organized process of cell death which takes place in the body under physiological and pathological conditions to eliminate redundant or metabolically exploited cells and to reduce cell numbers in the case of excessive proliferation. Apoptosis may be induced by external stimuli that activate various molecular death pathways, thus causing DNA degradation, damage to the protein structure and cell disintegration. The following processes can be observed in apoptized cells: chromatin hypercondensation and margination, cytoplasmic condensation, plasma membrane blebbing, and, eventually, division into apoptotic bodies that are quickly cleared by phagocytic cells (Jejurikar et al. 2006, Adhihetty et al.

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2008). Skeletal muscle represents a unique tissue with respect to apoptosis. Multinuclear muscle fibers seem to be relatively resistant to the harmful activity of pro-apoptotic factors. However, intensified apoptosis is observed in chronic heart failure, motor neuron disease and denervation, and leads to muscle atrophy, age-related sarcopenia, myopathies induced by genetic and inflammatory factors and by myotoxic drugs (Dupon-Versteegden 2005, Owczarek et al. 2005, Siu and Alway 2009). Apoptotic pathways in muscle fibers, the relevant control mechanisms and the level of initiator gene expression, differ in individual cases. The above factors are researched intensively as part of in vitro and in vivo studies.

Apoptotic signal transduction

Programmed cell death pathways may be activated by both external and internal stimuli. External pathways are activated by cell membrane death receptors which belong to the tumor necrosis factor receptor (TNFR) superfamily. Death receptors bind to ligands; for example, receptor TNFR1 binds to cytokine TNF alpha, receptor CD 95 (Apo-1/Fas) binds to the Fas ligand (Fas-L), and TNF-related apoptosis inducing receptors (TRAIL) R1, R2, R3 and R4 bind to the TNF-related apoptosis-inducing ligand (TRAIL/Apo2L) (Adhihetty and Hood 2003, Dupont-Versteegden 2005, 2006, Marzetti et al. 2010). Having recognized their ligands, these receptors trimerize and transduce the signal via the death domain (DD) to adaptor proteins, such as the TNF-R1 associated death domain (TRADD), Fas-associated death domain (FADD) or receptor interacting protein (RIP). The proteins receiving the signal from the receptor contain the death effector domain (DED) in the N-terminal part of the chain which recognizes and binds effector proteins, procaspase-8 or procaspase-10, in the death-inducing signaling complex (DISC). The activation of initiator caspase-8 leads to activation of the effector caspases i.e caspase-3, caspase-6 and caspase-7. Caspase-3 moves from the cytosol into the nucleus where it activates caspase-activated DNase (CAD), an endonuclease responsible for DNA fragmentation, damage to the nucleus and death of the mononuclear cell (Adhihetty and Hood 2003, Dupont-Versteegden 2005, 2006, Kaźmierczuk and Kiliańska 2010, Marzetti et al. 2010). Caspase-8 may also contribute to cell death by cleaving Bid, the anti-apoptotic Bcl-2 protein, into small active fragments referred to as truncated Bid (tBid). Truncated Bid is translocated from the cytosol to the mitochondria where it binds with Bax pro-apoptotic protein. It activates the mitochondrial apoptotic pathway by inducing the mitochondrial release of pro-apoptotic proteins (Adhihetty and Hood 2003, Adhihety et al. 2008, Marzetti et al. 2010). The mitochondria are involved in the apoptosis process. The mitochondrial intermembrane space contains various pro-apoptotic proteins, including cytochrome C, apoptosis-inducing factor (AIF), endonuclease G (Endo-G), second mitochondrial activator of caspase/direct IAP binding protein with low pI (Smac/DIABLO), and high temperature requiring protein A2 (Omi/Htra2), which induce apoptosis when translocated to the cytosol. The mitochondria are also the main producers of reactive oxygen species (ROS) which demonstrate direct and indirect pro-apoptotic activity (Marzetti et al. 2010). The mitochondrial apoptotic pathway is an intrinsic pathway which is stimulated mainly by extracellular chemical, genotoxic and stress-inducing factors as well as intercellular factors, such as increased levels of ROS and reactive nitrogen species (RNS) or disrupted Ca^{2+} homeostasis in the cytoplasm. The above factors stimulate mitochondrial outer membrane permeabilization (MOMP) and the opening of mitochondrial permeability transition pores (mtPTP) (Marzetti et al. 2010). MtPTP channels are protein complexes comprising three main components: a voltage-dependent anion channel (VDAC) located in the outer mitochondrial membrane (OMM), adenine nucleotide translocase (ANT) in the inner mitochondrial membrane (IMM), and cyclophilin D (CyPD) in the matrix, which interact with one another under the influence of various stimuli to form the mtPTP (Marzetti et al. 2010). The above leads to the state of mitochondrial permeability transition (MPT) where the IMM potential becomes dissipated, swelling of the mitochondria occurs, the proton motive force is lost, and subsequent uncoupling of oxidative phosphorylation and decreased ATP production ensue. The OMM eventually becomes disrupted, resulting in MOMP and the release of pro-apoptotic factors from the intermembrane space to the cytosol (Adhihety et al. 2008, Marzetti et al. 2008, Marzetti et al. 2010). The conformational status of mtPTP is regulated by proteins of the Bcl-2 family which are divided into three subfamilies: 1) anti-apoptotic proteins represented by Bcl-2, Bcl-X_L, Bcl-w, Mcl-1 and A1; 2) multidomain, pro-apoptotic proteins, including Bax and Bak; and 3) "BH3 domain only" proteins which are small pro-apoptotic proteins represented by Bik, Bad, Bim, Bid, Puma and Noxa. "BH3 domain only" proteins are sentinels for cellular damage and function. They translate the death signal to the multidomain Bcl-2 members by either activating pro-apoptotic Bax/Bak or inactivating anti-apoptotic proteins (Cheng et al. 2001, Chipuk et al. 2005, Martinez-Caballero et al.

2005). The balance among these three groups is finely regulated by a variety of gene products, including p53 and other apoptotic factors (Chipuk et al. 2005). Under normal conditions, Bcl-2 and Bcl-X_L block the activity of Bax and/or Bak. In apoptotic conditions, for example during the enhanced production of ROS and RNS, Bax translocates from the cytosol to the mitochondria, undergoes oligomerization, inserts into the OMM and probably interacts with ANT and VDAC, with mtPTP opening, and release of cytochrome C and other factors from the mitochondria (Marzetti et al. 2010). However, recent studies have shown that mtPTP opening is principally involved in necrosis and ischemia/reperfusion injury rather than intrinsic apoptosis (Baines et al. 2005, Nagawa et al. 2005). In addition to the mtPTP, mitochondria have a different alternative mechanism for the release of pro-apoptotic protein, referred to as mitochondrial apoptosis-inducing channels (MACs). The MAC is the high-conductance channel, it is absent from normal mitochondria, but it forms in the outer membrane during early apoptosis. This channel is small in diameter, and therefore, is only able to mediate the release of small proteins, such as cytochrome C (Martinez-Caballero et al. 2005). The Mac formation occurs without the loss of outer mitochondrial membrane integrity or depolarization, and it is regulated by the Bcl-2 family protein. Upon apoptotic stimulation, Bax and Bak translocate from the cytoplasm and oligomerize on the outer membrane of the mitochondria to form MACs independent of mtPTP components. On the other hand, Bcl-2-over-expression suppresses MAC activity and prevents cytochrome C release (Adhihety et al. 2003, Martinez-Caballero et al. 2005, Dejean et al. 2006). The expression of either Bax or Bak is necessary for cytochrome C release and MAC formation, but they are likely to be functionally redundant with respect to both processes (Cheng et al. 2001, Dejean et al. 2005, Martinez-Caballero et al. 2009). The pharmacological profile of MAC activity is still limited. However, dibucaine, propranolol and trifluoperazine have been identified in patch-clamp experiments as dose-dependent MAC inhibitors (Martinez-Caballero et al. 2005, Dejean et al. 2006). Nevertheless, MAC and the mPTP transient opening may act alone or in combination, depending on cell type and death stimulus, to relocate Bax to the mitochondria, remodel the mitochondrial cristae and maximize cytochrome C release to amplify the death signal (Scorrano and Korsmeyer 2003, Dejean et al. 2006). Upon release from the mitochondria, cytochrome C binds to apoptotic protease-activating factor-1 (Apaf-1), dATP, and procaspase-9 to form the apoptosome. Within this molecular complex, procaspase-9 is activated via homo-oligomerization, and it subsequently engages the effector caspase-3, an end-point in the caspase activation cascade (Adhihetty 2003, Marzetti et al. 2010).

The second intrinsic pathway, independent of mitochondria, involves caspase-12, an initiator caspase located at the cytoplasmic side of the endoplasmic reticulum (ER). Disturbances in intracellular Ca^{2+} homeostasis induce a calpain-mediated activation of caspase-12 and the subsequent activation of caspase-9 and -3, leading to apoptosis (Adhihetty 2003, Dupon-Versteegden 2005, 2006).

The cell also has an apoptosis mechanism that is independent of caspases and related to the mitochondrial release of the apoptotic inducing factor (AIF) and endonuclease G (Endo-G) which, when activated by poly(ADP-ribose) polymerase 1, calpains, cathepsins or Bax/Bak, are translocated from the mitochondrial intermembrane space to the nucleus. The above leads to chromatin condensation, extensive DNA fragmentation and apoptosis (Li et al. 2001, Adhihetty and Hood 2003, Dupon-Versteegden 2005, Artus et al. 2010). AIF is thought to be mainly responsible for chromatin condensation and cleavage of DNA into high molecular weight fragments; on the other hand, endonucleases Endo-G and CAD can produce low molecular weight (oligonucleosomal) DNA fragments (Susin et al. 1999, Zhang et al. 2003). As supported by current in vivo data, calpain-I appears to be the most important protease involved in the cleavage and the subsequent release of AIF from its membrane anchor in the IMM. Thus, dysregulation of Ca^{2+} homeostasis might be an important prerequisite step for the activation of mitochondrial calpain and of the AIF mediated pathway (Susin et al. 1999). The fact that numerous in vitro and in vivo studies have documented antioxidant inhibition of AIF-mediated cell death supports the notion that the intracellular ROS level positively regulates AIF cleavage and release (Bajt et al. 2006, Norberg et al. 2010). However, additional studies are needed to clarify the precise interaction between ROS and AIF-mediated cell death mechanisms. In addition to its nuclear effects, AIF also triggers mitochondria to release cytochrome C. AIF also plays a key role in mediating cell death in several pathological conditions, such as ischemic injury, neurodegenerative disorders and certain types of cancers (Norberg et al. 2010). Studies investigating the role of Endo-G have demonstrated that it often participates in drug-induced apoptosis, but is also involved in myofiber nuclear apoptosis in skeletal muscles undergoing atrophy due to disuse or ageing (Bajt et al. 2006, Dupont-Versteegden 2006).

Muscle-specific apoptotic distinctions

As a post-mitotic tissue, skeletal muscles can undergo apoptosis both in response to specific physiological stimuli and various pathological processes, and they are a unique tissue with respect to apoptosis. Firstly, muscle fibers are multinuclear cells, and the decomposition of one nucleus by apoptosis, referred to as nuclear apoptosis, does not lead to the death of the entire muscle fiber. However, nuclear apoptosis has a negative effect on gene expression in the surrounding cytoplasm, i.e. the myonuclear domain (MND), potentially leading to myofiber atrophy (Adhihetty et al. 2008, Liu et al. 2009). Secondly, skeletal muscles have the ability to induce changes in the mitochondrial pool, subject to the type of muscle fiber and in response to chronic alterations in muscle use or disuse (Adhihetty et al. 2008). Thirdly, skeletal muscles have two separate mitochondrial subfractions: subsarcolemmal (SS), which is located directly underneath the sarcolemmal membrane and accounts for approximately 20%, and intermyofibrillar (IMF), which accounts for around 80%, and is intermingled within the myofibrils. These mitochondrial subfractions possess different functional (e.g. respiration), compositional (protein and lipid) and biochemical (e.g. protein import) properties, which contribute to their capacities for adaptation. The SS subfraction is more labile than the IMF subfraction, displaying greater adaptive changes during chronic muscle use, disuse and in disease (Adhihetty et al. 2005). The results of research investigating mitochondrial fractions isolated from rat muscles have shown that IMF subfractions are more sensitive to apoptotic stimuli in comparison with the SS fraction, as demonstrated by higher expression of mitochondrial permeability transition pore components VDAC and CyPD with opening of the mtPTP and, consequently, higher release of cytochrome C and AIF in response to ROS (Adhihetty et al. 2005). Given the relatively high proportion of IMF mitochondria within a muscle fiber, this subfraction is probably the most important in inducing apoptosis when presented with apoptotic stimuli, ultimately leading to myonuclear decay and muscle fiber atrophy (Adhihetty et al. 2005).

The mitochondria are also the main site of ROS production which, in skeletal muscles, increases in response to contractile activity, ischemia/reperfusion, muscular dystrophies and muscle ageing (Siu et al. 2008, Huang and Hood 2009). Reactive oxygen species have both direct effects on apoptosis, as well as indirect effects via the activation of transcription factors. The potential of ROS to induce oxidative damage has significant implications for the cellular integrity of highly metabolic, long-lived and post-mitotic tissues such as brain, heart, and skeletal muscle (Jackson 2005, Huang and Hood 2009, Marzetti et al. 2010). In addition, the effect of ROS is exacerbated by its potential to induce mutations in mitochondrial DNA (mtDNA) which has no protective histones and has substantially fewer repair mechanisms than nuclear DNA. Mutations in mtDNA can lead to the synthesis of defective respiratory chain components, which may result in the impairment of oxidative phosphorylation, decreased ATP production and further ROS generation (Marzetti et al. 2010). In addition to mtDNA, ROS generated by the mitochondria can also directly damage proteins and lipids in the mitochondrial compartment. Oxidatively modified proteins within the electron transport chain (ETC) may actually produce more immediate harmful effects, such as increased uncoupling and decreased efficiency of oxidative phosphorylation (Marzetti et al. 2010). However, oxidative damage to IMM lipids, in particular cardiolipin, can lead to the disruption of membrane potential with the loss of the proton motive force and affect the activity of respiratory chain complexes, and can directly promote the release of apoptogenic factors from the mitochondria (Marzetti et al. 2010). Given the large share of mitochondria in the metabolism of muscle fibers, and the different sensitivity of mitochondrial subtractions to pro-apoptotic factors, the mitochondrial pathway seems to play a key role in skeletal muscle apoptosis.

Anti-apoptotic muscle components

Skeletal muscles have well-developed systems which protect them against oxidative damage, including both mitochondrial and cytosolic isoforms of superoxide dismutase (MnSOD and CuZNSOD, respectively), catalase and glutathione peroxidase enzymes, and a number of direct scavengers of ROS, including glutathione, taurine, vitamin E and ascorbate. In general, slow twitch, mitochondria-rich (type I) fibers have an increased content of protective systems in comparison with fast (type II) fibers (Jackson 2005, Marzetti 2010). Skeletal muscles and the heart muscle are characterized by the high expression of caspase-inhibiting proteins, which could suggest that those fibers have a unique resistance to apoptosis (Koseki et al. 1998). Among various identified apoptosis-regulating proteins, there is a group of endogenous proteins which suppress pro-apoptotic signaling. These proteins include the X-linked inhibitor of apoptosis (XIAP), the apoptosis repressor with caspase recruitment domain protein (ARC), and the Fas-associated death domain-like interleukin 1b-con-

verting enzyme-like inhibitory protein (FLIP), which are capable of selectively interacting with initiator or upstream capases, making them inoperative and thereby suppressing apoptosis (Adhihetty et al. 2008, Siu and Alway 2009). ARC and FLIP are highly expressed in muscle tissue. It is intriguing, but not evidently proved, that the known high resistance of mature muscle tissues to apoptosis is related to the abundant expressions of these two apoptotic suppressors (Koseki et al. 1998, Abmayr et al. 2004). It has been suggested that the apoptotic suppressive effects of ARC and FLIP are associated with their inhibiting interactions with selective caspases, in particular caspase-8 which is the initiator caspase in death receptor mediated apoptosis (Koseki et al. 1998, Abmayr et al. 2004). ARC, for example, inhibits cell death induced by the Fas pathway by binding to caspase-8 and rendering it dysfunctional (Abmayr et al. 2004). However, more recent findings indicate that ARC is able to interact with the pro-apoptotic Bax protein, and it exhibits an apoptosis suppressive effect by influencing mitochondria-mediated apoptotic signaling (Gustaffson et al. 2004). In regard to FLIP, it is suggested that its apoptosis-modulating effect is related to individual splice variants (e.g. protein isoforms), for instance, $FLIP_S$ vs. $FLIP_L$ or $FLIP_\alpha$ (Siu and Alway 2009). XIAP is a potent apoptosis-inhibiting member in the group of inhibitors of apoptosis proteins (IAP). In contrast to ARC and FLIP, the IAPs have both upstream (caspase-9) and downstream (caspase-3) inhibitory targets to prevent apoptosis by directly binding to the enzymatic site within these caspases. Relief of the inherent IAP suppression of caspases is achieved by the mitochondrial pro-apoptotic factors Smac/DIABLO and Omi/Htra2. These proteins are released from the mitochondrial intermembrane space upon an apoptotic stimulus, and they bind to the IAPs. This process eliminates IAP inhibitory activity, promotes caspase activation and leads to apoptosis (Adhihety and Hood 2003, Adhihety et al. 2008).

Heat shock proteins (HSPs) are also believed to play an important role in the prevention of apoptosis. Chaperone proteins inhibit apoptosis by limiting proteolytic maturation, activation and/or activity in fully functional caspases (Kaźmierczuk and Kiliańska 2010). HSP27, HSP60, HSP70 and HSP90 overexpression prevents the activation of those cysteine proteases in various types of cells following the accumulation of incorrect folding proteins or DNA damage resulting from various stressors, including ROS. A drop in the above expression levels due to the presence of antisense nucleotides or small interference RNA (siRNA) in the system, which inhibit HSP gene transcription, makes cells more sensitive to apoptosis (Kamada et al. 2007, Kaźmierczuk and Kiliańska 2010). The HSP70 protein in the cytosol is the best researched member of the HSP family to date, and it inhibits most stages of cell apoptosis (Adhihetty et al. 2008). HSP70 molecules decrease or completely inhibit caspase activation, and they restrict damage to the mitochondria and cell nuclei. HSP70 reacts with proteins Bcl-2 and Mcl-1 by blocking the translocation of pro-apoptotic protein Bax and inhibiting changes in the permeability of the OMM, thus blocking the efflux of cytochrome C and other mitochondrial pro-apoptotic factors (i.e. AIF, Smac/DIABLO or Endo-G) (Nylandsted et al. 2000, Stankiewicz et al. 2009). The reactions between HSP70 and cytosol members of the Apaf family (Apaf-1,-2,-3) inhibit the production of the apoptosome and the activation of procaspase-3 (Beere et al. 2000, Schmitt et al. 2007). HSP70 is also capable of interacting with and inhibiting the translocation of AIF and Endo-G from cytosol to the nucleus, thus preventing chromatin condensation, DNA fragmentation and cell death (Gurbuxani et al. 2003, Ruchalski et al. 2006, Lanneau et al. 2007). HSP70 has emerged as an important protein capable of inhibiting both the caspase-dependent (APAF-1) and the caspase-independent (AIF inhibition) apoptotic pathway. Because it is highly inducible by exercise, HSP70 might be an important factor attenuating apoptotic events in skeletal muscles as a result of death-evoking stimuli (Adhihetty et al. 2008). HSP90, another heat shock protein, may also play an important role in skeletal muscles. The protein has been found to interact with vimentin filaments, fibrillar proteins of the cytoskeleton in mesenchymal cells, protecting them against the proteolytic activity of caspases activated by apoptotic stimuli. The proteolysis of vimentin by caspases leads to disturbances in the filament structure which contribute to chromatin condensation, nucleus fragmentation and cell apoptosis (Lanneau et al. 2008). The appropriate expression of HSP90 could, therefore, play a key role in promoting muscle fiber growth and myocyte regeneration since vimentin filaments are present in the precursor cells of skeletal muscles, in maturing myotubes – in diminishing quantities, and in the cytoplasm of phagocytes which significantly contribute to the regeneration of damaged muscle fibers (Lanneau et al. 2008). The stabilization of vimentin, a cytoskeleton component is, therefore, required for the appropriate functioning of the entire cell, in particular during exposure to pro-apoptotic factors since it significantly affects the speed and quality of regeneration. Intriguing results were provided by experiments suggesting that lysosomes, which may act as death signal integrators in various types of cells, contain both HSP70 and HSP90 molecules (Nylandsted et al. 2004, Gyrd-Hensen et al. 2006). Lysosomes release cathepsins in response to apoptogenic

factors, such as TNF-α, ligand Fas, microtubule stabilizing agents, staurosporine, activated p53, oxidative stress or the downregulation of growth factors (Lenneau et al. 2008). Having entered the cytosol, these proteases catalyze changes in the OMM with the mtPTP opening (Nylandsted et al. 2004). The presence of both HSP90 and HSP70 molecules has been noted in lysosomes in various types of neoplasms and in healthy cells exposed to stress, where the above proteins inhibit cathepsin release into the cytosol. Therefore, high levels of HSP70 expression in lysosomes have a stabilizing effect on their plasma membranes, in particular in response to the adverse effects of chemical and physical factors (Nylandsted et al. 2004, Gyrd-Hensen et al. 2006).

Muscle autophagy and proteasome pathways

While a variety of different signal transduction pathways control the activation of apoptosis, the removal of myonuclear debris, damaged organelles and unfolded proteins is ultimately mediated by two general mechanisms, the ubiquitin-proteasome pathway (UPP) and autophagy (Adhihetty et al. 2008, Schwartz 2008).

Autophagy is a lysosomal degradation pathway that is essential for survival, differentiation, development, and homeostasis. Autophagy is activated as an adaptive catabolic process in response to different forms of metabolic stress, including nutrient deprivation, growth factor depletion, and hypoxia, to prevent cell death, but under specific conditions, it may constitute an alternative pathway to demise (authophagic cell death). It is not yet understood what factors determine whether autophagy is cytoprotective or cytotoxic, and whether cytotoxicity occurs as the result of self-cannibalism, the specific degradation of cytoprotective factors, or other so far undefined mechanisms. The most intuitive mechanism is self-cannibalism (Levin and Kroemer 2008, Maiuri et al. 2010). Skeletal muscles have a well functioning autophagy system that eliminates unfolded and toxic proteins as well as abnormal, dysfunctional and damaged organelles whose quantity increases in response to intensified catabolic processes in oxidative stress, denervation, inactivity, systemic diseases and ageing (Levin and Kroemer 2008, Sandri 2010). The autophagy system is responsible for generating double-membraned vesicles (autophagosomes) which engulf a portion of the cytoplasm, organelles, glycogen and protein aggregates which are then fused with lysosomes to create autolysosomes, where they are degraded and digested. Autophagy in skeletal muscles has unique features in comparison with other important metabolic tissues that show a transient and short (several hours) activation of autophagy during fasting. In contrast, skeletal muscles are characterized by a persistent generation of autophagosomes which continues for days. Prolonged autophagic induction requires the right balance between the inhibitors and the activators of the autophagic machinery. Some autophagy suppressors have been reported in skeletal muscles, including Runx1, which is upregulated in denervated muscle, phosphatase Jumpy or kinase Akt which inhibits autophagosome formation during fasting (Levin and Kroemer 2008, Maiuri et al. 2010). Upregulated autophagy transcription factors have also been identified, among them FoxO3, a critical factor in autophagy control in adult muscles. Expression of FoxO3 is sufficient and required to activate lysosome-dependent protein breakdown in cell cultures and in vivo. FoxO3 also regulates the expression of other autophagy-related genes, including LC3, Gabarap, Bnip3, VPS34 and Atg12. The p38hf MAPK pathway was recently found to regulate autophagy-related genes independently of Fox3 during oxidative stress (Sandri 2010). Although autophagy can independently influence the life and death decisions of a cell (by delivering a cytoprotective or a self-destructive effect), it is also intricately linked to apoptotic death pathways. Despite major efforts, the crosstalk between self-eating (autophagy) and self-killing (apoptosis) remains unclear, although the involvement of a functional and physical Bec1/Bcl-2 interaction has been suggested (Maiuri et al. 2010). Djavaheri-Mergny et al. (2010) proposed a model of apoptosis regulation by Beclin 1 and its fragments. Apoptotic stimuli which activate death receptor-mediated and mitochondria-mediated cell death pathways activate autophagy, a pro-survival mechanism. During sustained exposure to apoptotic stimuli, caspase-mediated cleavage of Beclin 1 generates fragments Beclin 1-N and Beclin 1-C which lose their ability to induce autophagy. The Beclin 1-C fragment translocates to mitochondria and sensitizes cells to apoptotic signals. This represents an amplifying loop mechanism for inducing massive apoptotic cell death. Factors which may facilitate the recruitment of Beclin 1-C to mitochondria and the enhancement of its permeabilizing activity have not been identified to date (Djavaheri-Mergny et al. 2010). The ubiquitin-proteasome pathway (UPP) is the major proteolytic process that is systematically activated in muscles in catabolic states such as cachexia, starvation, insulin deficiency and sepsis. Increased proteolysis is prevented only by proteasome inhibitors. The UPP has been recently demonstrated to degrade the myosin heavy chain (a major contractile protein) and telethonin, which plays a role in sarcomere integrity

(Attaix et al. 2008). The UPP is activated at the ubiquitination/deubiquitination and proteasome activity levels. The precise complex and interdependent signaling pathways and transcription factors (FoxO family) responsible for the activation of the UPP have been partially identified. Recent studies have also shown that FoxO3 activates not only the UPP, but also the transcription of autophagy-related genes (Attaix et al. 2008). The exact role of different muscle proteolytic systems and their respective substrates are still largely unknown (Attaix et al. 2008, Schwartz 2008). Studies in rodent models have established that uremia-induced, accelerated muscle protein catabolism involves proteolysis *via* the UPP (Mitch et al. 1996, Lecker et al. 2004). In fasting and, presumably, in other disease states, an identifiable function of accelerated proteolysis is to mobilize amino acids from dispensable muscle proteins to provide the organism with precursors for hepatic gluconeogenesis or for new protein synthesis (Mitch et al. 1996). Because the UPP serves many essential functions in cell regulation and homeostasis, its activation in these states must be highly selective and precisely regulated to avoid the unwanted removal of muscle proteins that are essential for cell function in muscles and other organs (Lecker et al. 2004). Vazeille at al. (2008) reported that the ubiquitin-proteasome and the mitochondria-associated apoptotic pathways are sequentially downregulated during recovery after immobilization-induced muscle atrophy. The activity of the UPP is first normalized, whereas apoptotic processes are later sequentially downregulated and normalized during recovery after immobilization. This sequential normalization of the Ub-proteasome-proteolytic and the mitochondria-associated apoptotic pathways suggests a possible cooperation to ensure proper muscle mass recovery (Vazeille et al 2008).

Myogenic cells

Tissue-specific stem cells, referred to as satellite cells, are an integral part of multinucleated myofibers. Muscle satellite cells are a population of undifferentiated mononuclear myogenic cells that are located under the basal lamina of muscle fibers. They are responsible for postnatal muscle growth, muscle plasticity and regeneration and repair of injured muscle. Satellite cells are normally quiescent, but they are activated in response to loading, muscle trauma or damage, prompting multiple rounds of proliferation and providing a population of myoblasts which subsequently withdraw from the cell cycle and express muscle-specific genes. Some myogenic cells fuse to form myotubes, whereas others serve as bridges between myofibers, aiding in their regeneration. Since myonuclei are postmitotic and incapable of undergoing cell division, muscle satellite cells provide the only known important source for adding new nuclei. Because of their fundamental role in muscle regeneration, robust mechanisms must exist to assure the survival of satellite cells within the context of a damaged muscle (Schultz and McCormick 1994). Once activated from quiescence, the amplifying progeny of satellite cells (myoblasts) are sensitive to apoptotic cell death as they proliferate and differentiate. The moment of satellite cell activation, within the first 24 h following myotrauma, is a critical period during muscle regeneration, during which satellite cells undergo an important series of molecular changes prior to cell division, while at the same time, in response to injury, intracellular reactive oxygen species are generated (Schultz and McCormick 1994). ROS is a key effector of DNA damage in myoblasts, while under conditions of transient oxidative stress, human satellite cells show decreased viability and become non-proliferative (Golding et al. 2007). Under normal conditions, catalase and glutathione transferase antioxidants expressed by quiescent satellite cells can protect against ROS. However, these defense systems become overwhelmed by excessive ROS production, as happens following injury and in various pathological conditions, thus increasing satellite cell sensitivity to apoptosis (Ji et al. 1998). Data from an *in vitro* investigation demonstrated that signaling via erbB2, a member of the epidermal growth factor (EGF) receptor tyrosine kinases family, provides an anti-apoptotic survival mechanism for satellite cells during the first 24 h of their activation, as they progress to a proliferative state (Golding et al. 2007). The four members of the ErbB receptor family (ErbB1 – ErbB4) are known as mediators of cell survival because they participate in the activation of anti-apoptotic factors, such as Ras, PI3-K, Akt, transcription factor STAT3 or Bcl-x/-2 (Danielsen and Maihle 2002, Golding et al. 2007). From these factors, STAT3 becomes potentially activated in satellite cells just after myotrauma, and it induces the transcription of anti-apoptotic Bcl-2/x and caspase inhibitors (Danielsen and Maihle 2002, Kami and Seneba 2002, Golding et al. 2007). While the majority of myogenic precursor cells (mpc) exit the cell cycle and undergo terminal differentiation during muscle repair or growth, roughly 30% of differentiating myoblasts undergo caspase-3-dependent apoptosis during differentiation. Interestingly, caspase-3 activity is also required for the initiation of myoblast differentiation into myotubes, but the exact mechanism leading to caspase-3 activation in this case remains elusive (Shaltouki et al 2007). In vitro experiments indicate that the activation of caspase-3 may

occur via the mitochondrial pathway, and that it is related to the release of cytochrome C and an increase in caspase-9 activity levels. Furthermore, the release of cytochrome C from the mitochondria is connected with the expression level of the pro-apoptotic Bcl2 family member PUMA (Shaltouki et al 2007). Finally, myoblasts rescued from apoptosis by either inhibition of elevated caspase-9 activity or silencing of PUMA are competent for differentiation (Shaltouki et al 2007). However, other investigators have suggested the possibility that another caspase-9-dependent, Bcl-xL-sensitive pathway is involved; nevertheless, they agree that caspase-3 and caspase-9 are not only agents of cellular disassembly, but they also help decide the fate of differentiating myoblasts (Murray et al 2008). The discovery of which caspase substrates are cleaved in differentiating myoblasts and which are not will be key to understanding how these cells survive the activation of the intrinsic apoptotic pathway and use this pathway to complete myotube formation (Murray et al. 2008). Skeletal muscles use recruited macrophages (MPs) to support post-injury regeneration, and it seems probable that MPs play a significant role in the regulation of myogenic cell death during regeneration. The MP influx is temporally correlated with the fading of myogenic precursor cell apoptosis during post-injury muscle recovery (Sonnet et al. 2006). In vitro studies have also shown that macrophages can stimulate mpc proliferation during regeneration through soluble mitogenic factors and prevent their apoptosis through direct cell-cell contacts involving adhesion molecules expressed by macrophages and myogenic cells (VCAM-1-VLA4, ICAM-1LFA-1, PECAM-1-PECAM-1 and CX3CLI-CX3CR1) (Chazaud et al. 2003, Sonnet et al. 2006). Moreover, it is possible that MPs can rescue differentiating mpcs from myoblast-fusion-associated apoptosis induced by endoplasmic reticulum stress, and they can help myotubes achieve structural stabilization during elongation through additional myoblast fusion (Sonnet et al. 2006).

Satellite cells also play a significant innate role in supporting muscle plasticity and adaptation, particularly in muscle enlargement/hypertrophy. Given that satellite cells regulate the adaptive and regenerative ability of skeletal muscles, it has been suggested that the quantitative depletion and the decline in the proliferative potential of muscle satellite cells contribute to the impairment of muscle regenerative capacity and the reduction of contractile function in muscular degenerative situations (Schultz and McCormick 1994, Jejurikar et al. 2002, Jejurikar et al. 2006). Jejurikar et al. (2002) demonstrated that in chronic muscle denervation, satellite cell numbers decline dramatically due to increased susceptibility to apoptotic cell death under denervation conditions. Subsequently, Jejurikar et al. (2006) demonstrated age-dependent differences in apoptotic susceptibility by indicating that satellite cells isolated from old rats show a greater response to pro-apoptotic agents by increasing apoptosis in comparison with young rats. The intensity of apoptotic processes in old animals was confirmed by an increased proportion of cells with activated caspases, intranucleosomal fragmentation of DNA and typical apoptotic phenotypes as well as cells with decreased amounts of protective protein bcl-2 (Jejurikar et al. 2006). The results of the above experiment suggest that programmed cell death may also contribute to the impairment of muscle regeneration with age (Jejurikar et al 2006).

The exact molecular and signaling mechanisms in the regulation of apoptosis in myogenic cell biology is still largely unknown, and further investigations are needed to completely understand the role of apoptosis in the depletion of satellite cells in various muscle pathologies (Siu and Always 2009).

Selected apoptosis-inducing factors in muscles

Although differentiated skeletal muscle seems to be apoptosis resistant, these cells can be induced to undergo apoptosis in response to various apoptosis-inducing factors, such as denervation, immobilization, ageing, mineralocorticoids (aldosterone), glucocorticoids (dexamethasone, betamethasone, triamcinolone), statins (simvastatin, lovastatin), chemotherapeutics (actinomycin D, staurosporine), local anesthetics (bupivacaine) and toxins (botulinum toxin A) (Narita et al. 2000, Irwin et al. 2002, Dupon-Versteegden 2005, Owczarek et al. 2005, Dirks and Jones 2006, Dirks-Naylor and Griffiths 2009, Tsai et al. 2010). These factors activate different apoptotic pathways, both in mature muscle fibers and in the population of myogenic cells; they are therefore used in studies investigating the possibility of programmed cell death regulation in various pathological and physiological states. One of such factors is dexamethasone, a glucocorticoid which induces apoptosis in muscle fibers by activating the mitochondrial pathway and the FAS receptor-dependent pathway. In experiments investigating glucocorticoid-induced apoptosis in rats, an increase in the expression levels of the Fas antigen, caspase-8, as well as Bax, Bad and Bid proteins was noted, whereas no differences were reported in the expression of anti-apoptotic proteins Bcl-2 and Bcl-X_L (Lee et al. 2001b, 2005). In other studies, an increase in the mitochondrial release of

cytochrome C and glucocorticoid-induced activation of caspase-9, caspase-3 and Apaf-1 protein was reported (Chauhan et al. 2001, Hoijman et al. 2004). In an experiment analyzing long-term dexamethasone exposure to rhabdomyosarcoma cells, Oshima et al. (2004) have demonstrated that dexamethasone induces mitochondrial dysfunction by affecting oxidative metabolism and ROS overproduction, which increases mitochondrial damage and activates mitochondrial apoptotic pathways. The above researchers have also shown that increased apoptosis applies to both proliferating and differentiating cells, the only difference being that the well-differentiated cells are apoptized at a later stage (Oshima et al. 2004). An in vitro study investigating the effects of dexamethasone on rat myoblasts revealed that the analyzed glucocorticoid is also capable of blocking the insulin-like growth factor (IGF-I), a hormone with anabolic properties that stimulates proliferation and regeneration and inhibits apoptosis (Singleton et al. 2000). Glucocorticoid-induced apoptosis in skeletal muscles has also been confirmed in an ultrastructural study where chromatin condensation across the nuclear membrane, folding of the sarcolemma and myofibril destruction in the area of damaged cell nuclei were observed (Lee et al. 2001a, 2005).

Simvastatin, a statin drug that is popularly used owing to its ability to lower plasma cholesterol levels, is yet another pro-apoptotic factor. Research results have revealed that statins are capable of inducing apoptosis in various cell types, including macrophages and skeletal muscle cells. An in vitro study has demonstrated that subject to the dose applied, statins can induce apoptosis in myoblasts, myotubes and well differentiated muscle cells (Jonson et al. 2004, Dirks and Jones 2006). Statins exert a pro-apoptotic effect by activating the mitochondrial pathway, as demonstrated by a drop in Bcl-2 protein levels without changes in the expression of Bax proteins, and an increase in the activity levels of cytochrome C, caspase-9 and caspase-3 (Jonson et al. 2004, Dirks and Jones 2006). The latest findings have also shown that statins impair mitochondrial functions by inhibiting the synthesis of ubiquinone, a vital element in the electron transport chain, and by decreasing ATP synthesis (Sirvent et al. 2008). The impairment of the mitochondrial electron transport chain leads to membrane depolarization, mtPTP opening and the escape of Ca^{2+} ions to the cytoplasm. It has also been found that statins directly affect ryanodine receptors (RyR), thus stimulating channel opening, inducing the escape of calcium ions from the endoplasmic reticulum, deepening the Ca^{2+} imbalance in the cell, damaging the contractile apparatus of muscle fibers and activating the mitochondrial apoptotic pathway (Sirvent et al. 2008).

Conclusions

The results of the reviewed studies into apoptosis induction suggest that the programmed death of muscle fibers is a complex process owing to the specific structure of muscle fibers and the presence of muscle precursor cells. Subject to the stimulating factor, various pathways of programmed cell death are activated in mature muscle fibers and in the population of undifferentiated tissue-specific stem cells. Mature muscle fibers seem to be resistant to pro-apoptotic stimuli, but apoptosis is intensified under certain conditions, such as chronic heart failure, motor neuron disease and denervation, ageing, myopathies induced by genetic and inflammatory factors as well as by myotoxic drugs. Programmed cell death also plays a vital role in regulating muscle fiber regeneration. The speed and intensity of this process is determined by the right balance between proliferation and apoptosis in muscle precursor cells, phagocytes, myotubes and young muscle fibers. More work is required to assess the regeneration of skeletal muscle fibers under exposure to pro-apoptotic factors and to determine the types of cells that are most sensitive to apoptosis. The issue of mechanisms regulating programmed cell death in skeletal muscles under physiological and pathological conditions is an interesting one, and it could be usefully explored in further research.

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