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The Proteolytic Systems of Muscle Wasting

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Abstract: Skeletal muscle represents one of the most plastic tissues of our body thanks to the presence of heterogeneous population of myofibers that confer to skeletal muscle the functional plasticity necessary to modulate its morpho-fuctional properties in response to a wide range of external factors. Thus, alteration in fiber type composition represents a major component in muscle wasting associated with muscle diseases. Several mechanisms have been proposed to account for the alteration in the morpho-functional properties of skeletal muscle under pathological conditions. In this review we will discuss the potential catabolic mediators of muscle atrophy and wasting.

Keywords: Autophagy, calpain, caspases, muscle atrophy, muscle wasting, ubiquitin-proteasome system.

INTRODUCTION

Skeletal muscle is composed of multinucleated myofibers, arising from mononucleated precursor cells. Under physiologic conditions, such as exercise, change in hormone levels, oxygen and nutrient supply, skeletal muscle is able to change their morpho-functional properties and to adapt to external factors. Moreover, after development, skeletal muscle maintains the capacity to regenerate in response to different stimuli, recapitulating many aspects of development. However, The functional performance and the regenerative capacity of skeletal muscle tissues declines during post-natal life and they are compromised in different diseases.

In this review we will discuss the general basis of muscle development and the molecular mechanisms associated with muscle atrophy and wasting.

MOLECULAR CONTROL OF MUSCLE DEVELOPMENT

Muscle development is the result of the combined action of several factors, emanating from neural tube, dorsal ectoderm and lateral mesoderm, ultimately leading to the generation of heterogeneous population of muscle fibers [1].

The myogenic determination factors (MDFs), namely Myf-5, MyoD, myogenin and MRF4, represent muscle-restricted members of transcription factors that orchestrate a specific program of muscle gene expression in a temporally and spatially distinct pattern (Fig. **1**) [2-9]. In particular, MyoD and myf-5 are required for the commitment of precursor proliferating cells to the myogenic lineage, whereas myogenin and MRF4 control the differentiation of committed cells into mature myofibers. The myogenic program is completed with the innervation of myofibers by motor neurons during a period known as maturation, at the end of which the functional performance and the heterogeneity of skeletal muscle are modulated by nerve activity. It has been also reported that the myogenic program is modulated at epigenetic level, suggesting the presence of molecular circuitry of transcriptional regulation based on modulation of chromatin structure by reversible acetylation of histone tails [10].

Although the role of MDFs during embryonic development has been extensively investigated, less is known about their role during post-natal life. MyoD, myf-5 and myogenin expression decline during the first week of post-natal life, whereas MRF4 is predominantly expressed in adult skeletal muscle. However under certain conditions, such as aging, diseases, and injury, the re-activation of MDFs expression recapitulate the myogenic program [11-13].

MOLECULAR ORGANIZATION OF SKELETAL MUSCLE TISSUE

The cytoskeletal proteins, which can be subdivided in four major groups: the contractile sarcomeric, the intra-sarcomeric, the peri-sarcomeric and the sub-sarcolemmal proteins [14], play important role in the maintenance of muscle integrity and in the modulation of several signal transduction pathways; thus alteration in one or more cytoskeletal-associated components leads to muscle diseases (Fig. **2**).

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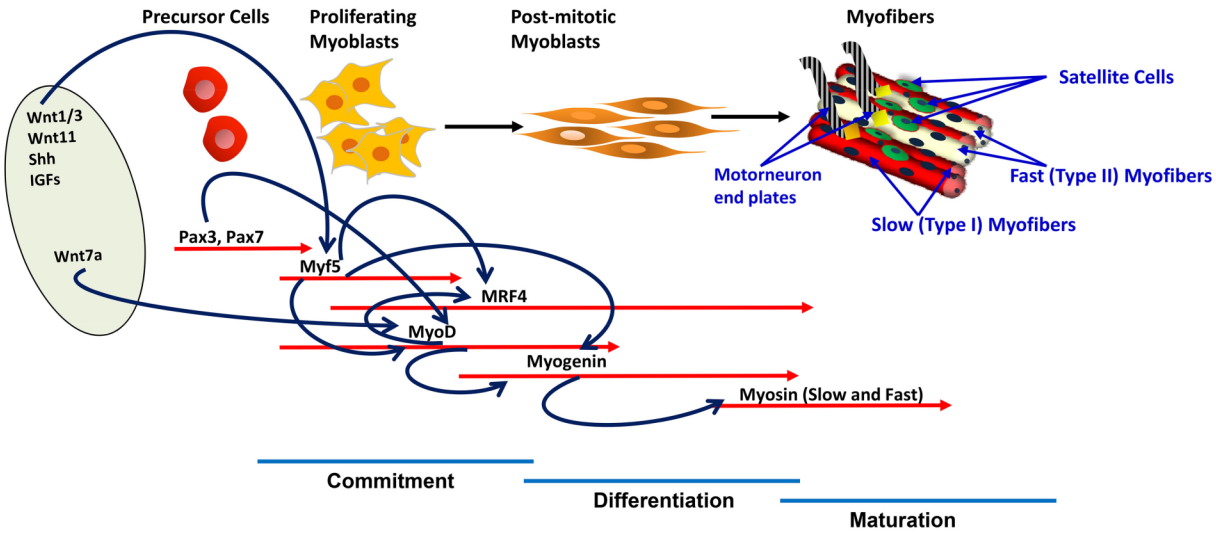

Fig. (1). Schematic representation of muscle formation during embryonic development. Developmental myogenesis occurs in three distinct stages: commitment, differentiation and maturation. Skeletal muscles are derived from somites which receive signals from the neighboring tissues, which in turn induce the activation (blue arrows) of muscle-regulatory factors (MyoD, Myf5, myogenin, and MRF4). Sonic hedgehog Shh (Shh) (from the notochord) and Wingless-related integration site (Wnt) Wnt1/3, Wnt11 and insulin-like growth factors (IGFs) (from dorsal neural tube) signaling have been shown to regulate the expression of Myf5. Pax3 and Myf5 independently regulate MyoD expression, whereas Myf5 regulates the transient expression of MRF4. Myf5 and MyoD independently activates the expression of Myogenin, which promotes the expression of Myosin (modified from [13]).

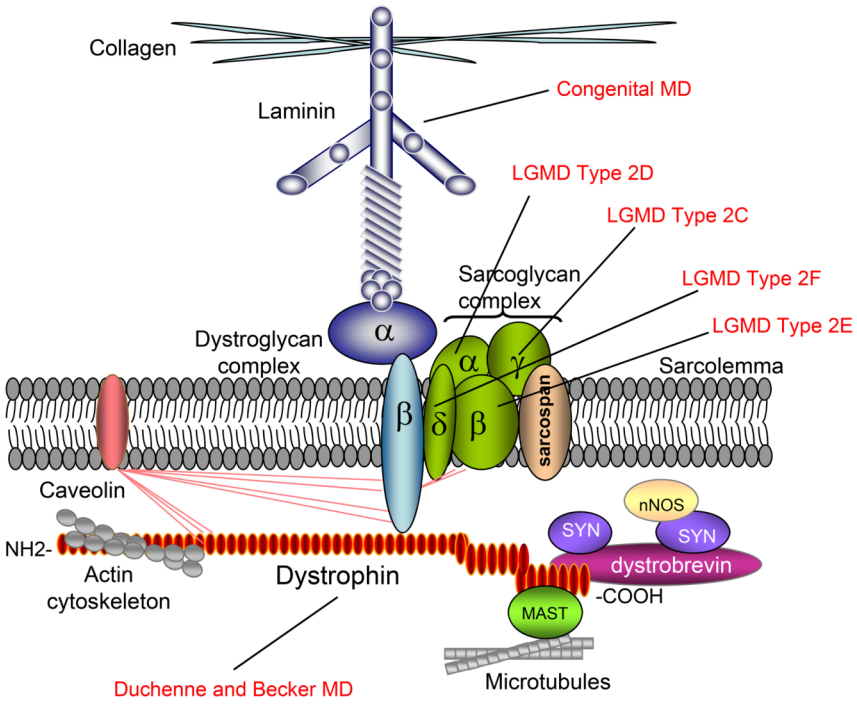

Fig. (2). The molecular organization of cytoskeleton protein. The dystrophin protein is associated with the dystroglycan complex and connects the cytoskeleton of a muscle fiber to its surrounding extracellular matrix. Mutations in various members of these proteins are associated with different muscle disorders (text in red).

Actin and myosin myofilaments belong to the contractile sarcomeric cytoskeleton and represent the functional core of muscle contraction; titin, nebulin, tropomyosin and actinin are classified as intra-sarcomeric protein; desmin-intermediate filament is a component of the peri-sarcomeric cytoskeleton, whereas the sub-sarcolemmal cytoskeleton includes sarcolemma-associated protein, such as dystrophin, vinculin, integrins, α-actinin, and ankyrin. Among the proteins that

are associated with the plasma membrane, the dystroglycan complex, formed by the subunits α and β dystroglycan, bind to the dystrophin protein and plays a critical role in the connection of the cytoskeleton of a muscle fiber to its surrounding extracellular matrix [15] (Fig. **2**).

Other structural components of skeletal muscle are the caveolins, the principal components of the caveolae, vesicular invaginations of the plasma membrane, which participate to trafficking events and signal transduction processes [16]. Among caveolin, caveolin-3 is localized to the sarcolemma where it forms a complex with cytoplasmic signaling molecules (G-proteins and Src-like kinases), dystrophin and its associated glycoproteins, such as alpha-sarcoglycan and beta-dystroglycan [17] (Fig. **2**). It has also been demonstrated that deficiency of caveolin-3 is causally linked to limb girdle muscular dystrophy (LGMD) [18].

The structural integrity of complex cytoskeleton molecular organization is therefore a prerequisite for the efficient mechanical function of skeletal muscle; thus alterations in cytoskeletal organization lead to muscle wasting and diseases.

THE MOLECULAR MECHANISMS OF MUSCLE WASTING

A general feature associated with several pathologic conditions is the loose of adaptability of skeletal muscle leading to wasting, a process in which the delicate balance between anabolic and catabolic process is impaired [19, 20]. Among different signalling, Calcium (Ca2+) is an important intracellular messenger, controlling numerous cellular processes, including proliferation, cell growth, differentiation, and gene transcription, through the activation of so called "toolkit", which comprises an array of signalling, homeostatic and sensory mechanisms [21]. However, defects in signalling "toolkit" can compromise the functional performance of muscle and activate proteolytic systems, leading to muscle wasting [22]. For example, it has been demonstrated that in Duchenne muscular dystrophy, the absence of dystrophin causes alterations in intracellular Ca2+ leading to an imbalance between muscle protein synthesis and protein degradation, culminating in necrosis, fibrosis, and shift in fiber content [23]. In other pathologic conditions, such as sarcopenia, the accumulation of free radicals promotes an increase in calcium concentration, which induces the activation of proteolytic systems with the consequence of an increase in protein degradation and reduction in protein synthesis [24].

Among proteolytic systems calpain-, ubiquitin- caspase- and autophagy-mediated protein and organelles degradation are the principal pathways activated in several pathologies, leading to myofiber degeneration, wasting and impaired muscle regeneration.

THE CALPAIN PATHWAY

Calpains, which have been identified in many organisms [25], are calcium-activated cysteine proteases characterized by the presence of two subunits, an 80kDa large subunit that contains protease activity and a 30kDa smaller subunit, functioning as a regulator of calpain activity [26]. Based on Ca^{2+} concentration dependence, two ubiquitous calpain isoforms, μ and m, are well characterized, and are commonly referred as the ubiquitous/conventional/typical calpains. Among the different subtypes, calpain 3 is predominantly expressed in skeletal muscle and it maintains proteolytic activity at physiological Ca^{2+} [27]. More recently, it has been demonstrated that calmodulin (CaM), a known transducer of the calcium signal, binds and facilitates Calpain 3 autolytic activation, providing additional insights into the mechanisms of Calpain 3 regulation in skeletal muscle [28]. At physiologic levels, calpains participate in many cellular processes, including cytoskeletal remodeling [29], cell mobility [30], myofibril maintenance [31], signal transduction [32] cell cycle progression [33], regulation of gene expression [34], apoptosis [35], and long term potentiation [36]. Calpains are also activated by several stimuli in which intracellular Ca^{2+} homeostasis is affected, causing sarcomeric alterations [37], mitochondrial swelling, sarcoplasmic reticulum vacuolization [38, 39] and disruption of the contractile tissue [40] (Fig. **3**).

Calpains are preferentially localized in the Z disk of the sarcomere [41] where they initiate the proteolytic cleavage of muscle proteins that anchor the sarcomere, including titin, nebulin, desmin, filamin, troponin and tropomyosin, causing the complete disassembly of myofibrils and loss of the Z disk [42, 43]. Fodrin, the non-erythroid spectrin protein and a major component of the cortical cytoskeleton of most eukaryotic cells including muscle cells, is another molecular target of calpains [42]. Cleavage of fodrin accompanies apoptosis induced by treatment of cells with staurosporine, glucocorticoid, or synthetic ceramide [43, 44], all factors that affect muscle physiology and myofibers survival.

The activity of calpains is regulated by the endogenous inhibitor calpastatin which prevents both enzyme activation and expression of catalytic activity [45] (Fig. **3**). The interactions between calpastatin and calpains are modulated by intracellular Ca^{2+}concentration [46].

In contrast to other proteolytic systems, calpains cleave target proteins at specific sites, leaving large polypeptide fragments with altered physiological properties. In addition, post-translational modifications provide a mechanism of "marking" specific proteins for calpain degradation, as for troponin T and I that are phosphorylated by protein kinase A and C, which alter the sensitivity of the protein to calpain degradation [47]. The activity of calpains is also associated with neutrophil accumulation during exercise [48] and can cause proteolysis and muscle fiber degradation in Duchenne and Becker muscular dystrophies (DMD/BMD) [49], suggesting a role for calpains in muscle injury and in the pathogenesis of muscle diseases. Of interest is the observation that calpain3 is associated with limb-girdle muscular dystrophy type 2A [50], and might control myoblast fusion and maturation, suggesting a role in muscle function [51].

More recently, two sarcomeric tropomodulin (Tmod) isoforms, Tmod1 and Tmod4, have been characterized, which represent novel proteolytic targets of m-calpain [52]. The levels of m-calpain resulted elevated in dystrophic soleus muscle of mdx mice and were associated with loss of Tmod1 from the thin filament pointed ends, suggesting that Tmod proteolysis, by m-calpain, represents a novel mechanisms that may contribute to DMD pathology [52].

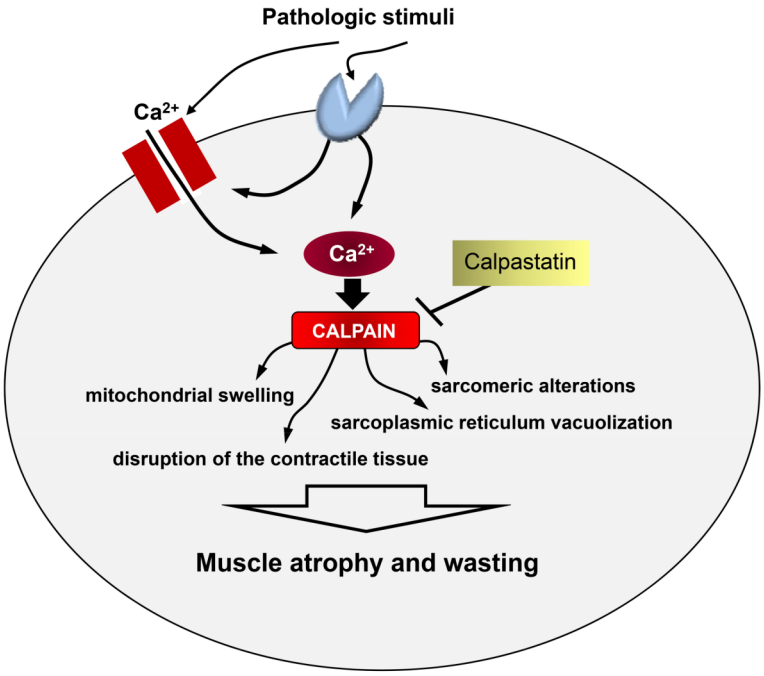

Fig. (3). Schematic representation of the calpain-mediated alteration in skeletal muscle leading to atrophy and wasting. Calpains are Ca^{2+}-activated cysteine proteases, which might target different intracellular components, causing disruption of the contractile tissue, mitochondrial swelling, sarcoplasmic reticulum vacuolization, and sarcomeric alterations, leading to muscle atrophy and wasting. Calpastatin represents the negative regulator of calpain activity.

In other reports, the catabolic activity of calpains, along with the ubiquitin-proteasome pathway, has been associated with muscle atrophy induced by chronic hypobaric hypoxia and with sarcopenia [53].

Sarcopenia is a pathologic consequence of aging, associated with loss of muscle mass and function. It has been demonstrated that sarcopenia is greatly reduced by muscle-specific overexpression of calpastatin. Of note, the activity of calpain can be regulated by nitric oxide (NO) through S-nitrosylation. Interestingly, Samengo *et al* [54] demonstrated that neuronal nitric oxide synthase (nNOS), the primary source of muscle NO, is loss during aging, whereas expression of a muscle-specific nNOS transgene restores calpain S-nitrosylation in aging muscle and prevents sarcopenia.

Other reports provided additional insights into the pathogenic role of calpain in sarcopenia. One of the leading mechanistic theories for aging is the oxidative damage hypothesis, based also on the evidences that age-related changes accelerate with elevated oxidative stress [55, 56]. It has been demonstrated that calpains activate the degradation of oxidated myofibrillar proteins [57], suggesting a mechanistic link between oxidative stress and accelerated myofibrillar proteolysis in disuse muscle atrophy [57, 58]. In addition, it has been reported that the expression of a calpastatin transgene, the endogenous inhibitor of calpains, attenuates muscle wasting associated to muscle disuse and prevents the shift from slow to fast of muscle fiber type in muscle unloading [59].

Collectively these studies demonstrate that calpains play a key role in both muscle homeostasis and diseases. Future study will be needed to test the feasibility of Calpains in gene therapeutic applications and to identify their *in vivo* substrates.

THE UBIQUITIN-PROTEASOME SYSTEM

One of the signal transduction pathways that mediates the turnover of muscle protein is the ubiquitin-proteasome system, which is also over-activated in several conditions leading to muscle wasting (e.g. glucocorticoid treatment, sepsis, fasting, cancer, and acidosis) [60-63]. The pathway involves an enzymatic cascade starting with the conjugation of protein substrates with ubiquitin and terminating with the degradation of targeted protein to small peptides and amino acids (Fig. **4**). Activated ubiquitin is then transferred by E1 to a carrier protein E2 (ubiquiting-conjugating enzyme); in the last step the ubiquitin-protein ligases E3 recognize the protein which will be ubiquitinated and catalyze the covalent interaction between the carboxyl group of ubiquitin and the ε-amino group of lysine in the protein substrate (Fig. **4**). These reactions are repeated to form an ubiquitin chain and finally the ubiquitin-conjugated proteins are transferred, in an ATP-dependent reaction, in the 26S proteasome complex where the proteins are degraded (Fig. **4**). The ubiquitin itself is then released and reused for a new enzymatic reaction (Fig. **4**).

It has been demonstrated that several pathologic conditions, associated with muscle atrophy, over-activate an "atrophy program" in which the ubiquitin-proteasome pathway represents the major player [64-66]. The muscle-specific ubiquitin-ligases, atrogin-1 (MAFbx) and Muscle RING Finger 1 (MuRF1) are up-regulated in muscle atrophy [67-69]. Indeed, knockout animals deficient in either MAFbx or MuRF1 are resistant to atrophy [68], whereas their overex-

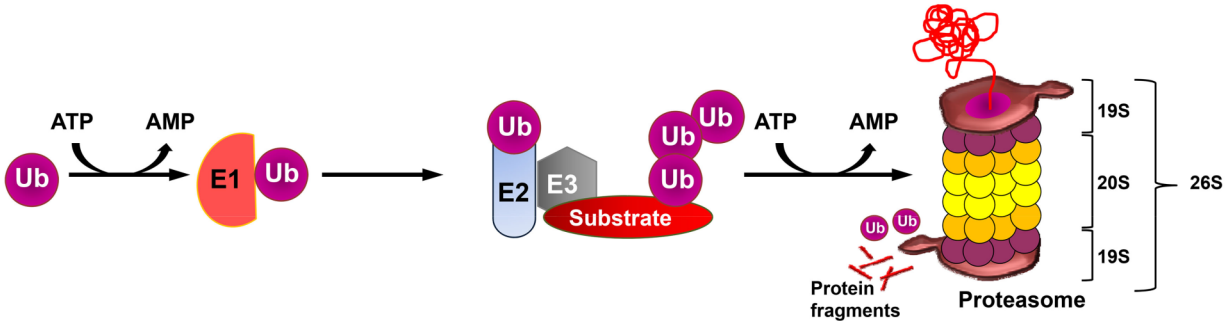

Fig. (4). Schematic representation of the ubiquitin-mediated protein degradation. Ubiquitin is activated by the ubiquitin-activating enzyme-E1 and then transferred to a ubiquitin-conjugating enzyme-E2. The E2 enzyme and the protein substrate both bind to the ubiquitin-protein ligase-E3. The protein substrate becomes polyubiquitinated; the polyubiquitin chain functions as a signal to target the polyubiquitinated protein substrate to the 26S proteasome, which represents a multicompartmentalized protease formed by two subunits: 20S and 19S, for degradation, generating short peptides and free ubiquitin that can be further reused.

pression produce atrophy and disrupts the integrity of sarcomeric structure and alter the components of thick filaments [69]. Several factors affect muscle homeostasis [69]; among these, glucocorticoids stimulate muscle protein breakdown, activating the ubiquitin-proteasome proteolysis and promoting muscle atrophy [69]. The reduction in anabolic factors, such as the insulin-like growth factors-1 (IGF-1), represents another potent stimulus for the activation of ubiquitin-proteasome pathway and therefore protein degradation. IGF-1 has been implicated in many anabolic pathways in skeletal muscle [70-73] and, considering that its expression declines with age, it has been suggested that this down regulation is likely to be causally linked to the progress of muscle atrophy in senescence, limiting the ability of skeletal muscle to sustain regeneration and repair [72-76]. It has been reported that the decreased activity of the IGF-1/PI3K/AKT signaling pathway can lead to muscle atrophy [77], via the activation of the "atrophy program". One downstream target of the IGF-1/PI3K/AKT pathway is the Forkhead box O (FoxO) class of transcription factors [78, 79]. AKT blocks, by phosphorylation, the function of FoxO factors, leading to their sequestration in the cytoplasm [80]. Reduction in anabolic factors, including IGF-1, causes the inhibition of the PI3K/AKT pathway. In such conditions, the dephosphorylated active form of FoxO factors enter to the nucleus where transactivate the expression of atrogin-1 and MuRF1 [79]. Of note, kinases downstream of AKT, including GSK3β, as well as the MEK and calcineurin systems, which have been implicated in the regulation of muscle fiber size [77, 81, 82] do not have direct roles in the regulation of atrogin-1 expression [79]. More recently, two additional targets of FoxO activity have been identified: SMART (Specific of Muscle Atrophy and Regulated by Transcription) and MUSA1 (muscle ubiquitin ligase of SCF complex in atrophy-1) [83, 84]. MUSA1 is a novel ubiquitin ligase that plays a critical role for the induction of muscle atrophy during denervation and fasting [81, 82]. Inhibition of MUSA1, by RNAinterference, prevents muscle atrophy, whereas excessive MUSA1 induction exacerbates muscle loss, causing muscle cachexia [81]. It has been also demonstrated that FoxO3 regulates another proteolytic pathways, namely autophagy (discussed below), coordinating the proteasomal-dependent removal of proteins with the autophagy-dependent clearance of organelles.

THE CASPASE PATHWAY

Caspases represent a central component of the proteolytic system and apoptotic machinery dysregulated in several diseases, including cancer and muscular dystrophies [85]. Caspases are all expressed as inactive proenzymes and are activated after cleavage at specific aspartate residues, generating the active product having lower molecular weight [86]. Caspases are classified in two major groups: the initiator caspases, which include caspase-2, -8, -9 and -10, that initiate the proteolitic cascade, and the effector caspases that include caspases-3, -6 and -7. Once activated, the effector caspases are responsible for the proteolytic cleavage of a broad spectrum of cellular targets, which ultimately leads to cell death [86] (Fig. **5**). While the initiator caspases are auto-activated, the activation of an effector caspase is carried out by an initiator caspase. The activation of caspases can be triggered by either extrinsic death stimuli in which specific ligands triggers a cascade of events leading to the activation of initiator caspases, or alternatively by intrinsic death stimuli inducing mitochondrial release of cytochrome c and formation of the apoptosome upon cytochrome c binding to Apaf1 (Fig. **5**) [85, 86].

A key role of caspases is to inactivate proteins that protect living cells from apoptosis. It has been reported that the cleavage of ICAD [87, 88], an inhibitor of the caspase-activated deoxyribonuclease (CAD), leads to DNA fragmentation. In non-apoptotic cells, CAD is present as an inactive complex with ICAD. Under apoptotic stimuli, caspases inactivate ICAD leaving CAD free to fragment DNA. Caspases also cleave structural proteins of the nucleus and cytoskeleton. Proteolysis of lamins promotes the destruction of nuclear lamina and allows chromatin condensation [89, 90], whereas the cleavage of desmin, by caspase 6, generates a small product (N-desmin) that activates apoptotic pathways [91]. Similarly, the caspase-dependent cleavage of other proteins, such as gelsolin, which controls cell motility and morphology, or β-catenin, generates fragments that might alter cell-cell contacts and cell-matrix focal adhesions, DNA repair, mRNA splicing, and DNA replication, promoting apoptosis [85, 92, 93].

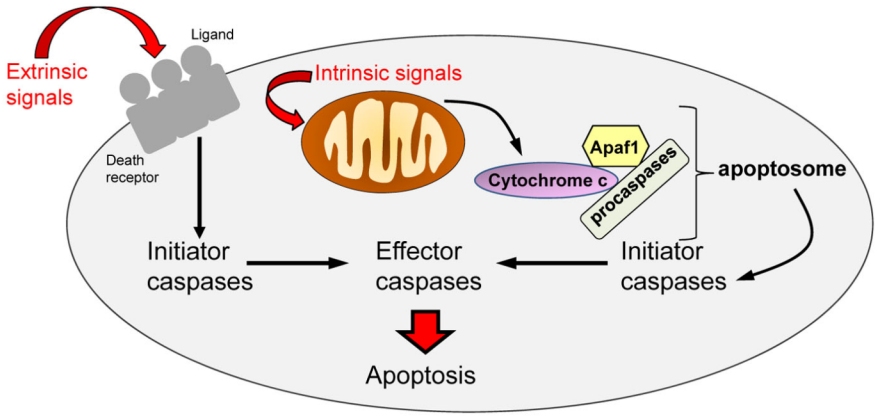

Fig. (5). Schematic representation of the caspase-mediated cell death. Caspases can be activated by either extrinsic or intrinsic death stimuli. Extrinsic death signals promote the activation of initiator caspases, which in turn induce the effector caspases, responsible for the proteolytic cleavage of cellular targets. Intrinsic death stimuli, such as the cytosolic release of cytochrome c in response to mitochondrial damage. The cytochrome c binds to Apaf1 and promotes the recruitment of procaspases to form the apoptosome, resulting in the activation of initiator caspases and then to the induction of effector caspases and subsequently to cell death.

Caspase-mediated apoptosis is also associated with different muscular diseases, including Duchenne and facioscapulo-humeral dystrophies [94]. One of the proposed mechanisms that mediates the activation of caspase pathway in muscular dystrophy is the endoplasmic reticulum (ER) stress, caused by calcium dysregulation, hypoxia/ischaemia and oxidative stress [95-98]. ER stress causes alteration in protein folding; thus unfolded and misfolded proteins accumulate in the ER lumen, triggering the apoptotic pathway (reviewed in [99]). Of note, ER stress activates the initiator caspases-12 and-4, which localized to the cytoplasmic face of the ER membrane [100, 101] and in turn activate caspase-9 and caspase-3, leading to apoptosis [102, 103]. Activated caspase-12 can also translocates to the nucleus, and may carry out apoptotic events directly [104], whereas deleting caspase-12 preserves muscle function in the mdx dystrophic mouse model, resulting in recovery of both specific force generation and resistance to eccentric contractions [105].

Caspases are also involved in the catabolic processes of skeletal muscle associated with cancer cachexia and in the inhibition of muscle differentiation [106]. The inflammatory cytokines tumor necrosis factor-alpha (TNFα) induces muscle proteolysis associated with cancer and negatively modulates skeletal muscle differentiation [107-109]. TNFα can activate NF-kB, one of the central players implicated in muscle wasting in different pathologic conditions [110], and can inhibit muscle differentiation upon the expression of PW1 [109, 111]. It has been suggested that the Jak2/Stat3 singnaling pathway is involved in cancer cachexia [112-114]. Accordingly, molecular inducers of this pathway, such as IL-6, oncostatin M, leukemia inhibitory factor (LIF), and ciliary neurotrophic factor (CNTF), have each been reported to cause loss of muscle mass [115, 116]. Moreover, pharmacological and/or genetic inhibition of Jak/Stat3 signaling, or IL-6 activity, counteract cancer-associated cachexia, age-induced sarcopenia, and muscular dystrophy, stimulating muscle regeneration [112, 113, 117-120]. Silva and co-workers [121] have demonstrated that p-Stat3, by binding to the caspase-3 promoter, stimulates caspase-3 transcription. It has been proposed that caspase-3 promotes muscle protein losses in two ways: it first cleaves the complex structure of actomyosin and myofibrillar proteins to produce substrates for the ubiquitin proteasome system and then caspase-3 stimulates 26S proteasome activity by cleaving some regulatory subunits of the 19S particle [121-123]. Inhibition of Stat3 activation suppresses caspase-3 and the ubiquitin-proteasome system, leading to preservation of muscle mass in cancer cachexia [121].

Interestingly, the discovery that the activities of caspase-3 and the ubiquitin-proteasome system work together to stimulate muscle wasting indicates how increased proteolysis can be achieved while maintaining specificity [124].

THE AUTOPHAGIC PATHWAY

Autophagy is a critical mechanism for all eukaryotic organisms, since it ensures specific cytosolic rearrangements needed for proliferation, death, and differentiation during embryogenesis and postnatal development [125]. Autophagy is activated as an adaptive catabolic process in response to different metabolic stress, including fasting, growth factor depletion, hypoxia, denervation, etc. Whereas basal level of autophagy is essential for physiological turnover of old and damaged organelles, the activation of autophagic pathway beyond a certain threshold may promote cell alterations by causing the collapse of cellular functions as a result of cellular atrophy [126, 127].

We recently demonstrated that autophagy plays a dominant role in the promotion of muscle atrophy associated with local alteration in the activity of the antioxidant enzyme SOD1 [128, 129]. In particular, oxidative stress might activate the transcription factor FoxO3 that in turn promotes the transcriptional activation of autophagy-related genes, such as LC3, cathepsin L and Bnip3. In addition, our study documented how the T-tubule might be the potential donor of membranes forming the autophagic vesicles. In the auto-

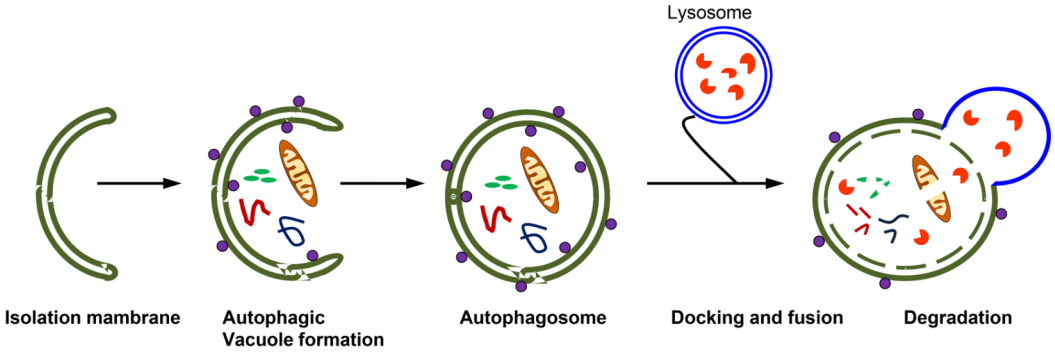

Fig. (6). Schematic representation of the autophay-mediated degradation. Autophagy involves the formation of a cytosolic double-membrane vesicle, an autophagosome, which sequesters and degrades components of the cytoplasm. The formation of autophagosome occurs in different steps: a portion of cytoplasm is first enclosed in a double structure, that surrounds cytoplasmic cargo, forming an autophagic vacuole that docks and fuses with the lysosome. The lysosomal hydrolases degrade proteins, organelles and cellular components. The purple circle in the figure represents LC3.

phagy-lysosome system, small ubiquitin-like molecules (among which LC3) are transferred to isolation membranes to trigger their growth and commitment degradation of the inner membranes of the autophagosome and of the engulfed proteins [130-133] (Fig. **6**).

The search for muscle markers associated with protein breakdown has revealed that under pathologic conditions the increased expression of lysosomal cystein endopeptidase cathepsin L and LC3 is associated with skeletal muscle wasting [134-136]. In fact, it has been reported that cathepsin L expression is up-regulated in skeletal muscle of animals bearing sepsis and cancer and in rat treated with the glucocorticoid analogue dexamethasone [134]. On the other hand, cathepsin L expression decreases in tumor-bearing animals treated with an inhibitor of tumor necrosis factor-alpha (TNF-α). These studies suggest that cathepsin L expression represents an early marker of muscle wasting associated with the activation of proteolytic pathways.

As mentioned above, FoxO3 is necessary and sufficient for the induction of autophagy in skeletal muscle under different pathologic conditions [79, 137]. Interestingly, a major effector of FoxO3-mediated autophagy in skeletal muscle is Bnip3, since Bnip3 expression is induced by FoxO3, whereas Bnip3 knockdown blocks FoxO3-induced autophagy [135, 137].

An alternative pathway that has been described to induce autophagy independently of FoxO3 is the p38 αβ MAPK [138], although the specific transcription factors downstream of p38MAPK remains unclear.

Recently, we published a set of guidelines for the examination of macroautophagy and related processes [139]. This because there is a difference between measurements that monitor the numbers or volume of autophagic elements vs. those that measure flux through the autophagy pathway. Thus, the appearance of more autophagosomes does not necessarily equate with more autophagy. In fact, in many cases, autophagosomes accumulate because of a block in trafficking to lysosomes without a concomitant change in autophagosome biogenesis, whereas an increase in autolysosomes may reflect a reduction in degradative activity [139].

CONCLUSIONS

The continual synthesis and degradation of cell proteins are the result of normal intracellular metabolism and represent an important homeostatic function of muscle tissue. Muscle wasting, in contrast, is a process in which the delicate balance between anabolic and catabolic process is impaired. In this review we described the major proteolytic systems that are over-activated in muscle pathologies, although the identification of the exact signaling cascades that regulate muscle wasting is only at the initial step. Different studies indicate that the development of muscle wasting is a multifactorial process and believed to be the result of both intrinsic factors, involving changes in molecular and cellular levels, and extrinsic ones, such as nutrition and exercise. Modulation of these pathways therefore comprises an attractive target for drug intervention. Therefore, it is of fundamental importance to gain greater knowledge about the molecular processes controlling the debilitating conditions to find effective countermeasures.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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