The Molecular Mechanisms of Calpains Action on Skeletal Muscle Atrophy

Jiaru HUANG¹, Xiaoping ZHU²

¹Ningxia Medical University, Yinchuan, Ningxia, China, ²Department of Respiratory Diseases, YangPu Hospital of Tongji University, Shanghai, China

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Summary
Skeletal muscle atrophy is associated with a loss of muscle protein which may result from both increased proteolysis and decreased protein synthesis. Investigations on cell signaling pathways that regulate muscle atrophy have promoted our understanding of this complicated process. Emerging evidence implicates that calpains play key roles in dysregulation of proteolysis seen in muscle atrophy. Moreover, studies have also shown that abnormally activated calpain results muscle atrophy via its downstream effects on ubiquitin-proteasome pathway (UPP) and Akt phosphorylation. This review will discuss the role of calpains in regulation of skeletal muscle atrophy mainly focusing on its collaboration with either UPP or Akt in atrophy conditions in hope to stimulate the interest in development of novel therapeutic interventions for skeletal muscle atrophy.

Key words
Cell signaling ● Calpains ● UPP ● Akt ● Muscle wasting

Introduction
Skeletal muscle atrophy, defined as the unintentional loss of 5-10% of muscle mass (Kotler 2000), is present in numerous pathologies such as cancer (Sarah et al. 2011), sepsis (Smith et al. 2008), neuromuscular disorders (Park et al. 2012, Cho et al. 2015) and diabetes (Yoshikawa et al. 2000). Muscle atrophy can also occur in the absence of diseases due to prolonged periods of muscle inactivity (Sieck and Mantilla 2008, Salazar et al. 2010), which can contribute greatly to reduced life quality and to increased mortality. Thus understanding the molecular contributors to muscle atrophy is a prerequisite for development of therapeutic strategies to improve clinical outcomes on muscle atrophy and reduce the burden on health care systems.

Many molecular events contribute to muscle atrophy, including events like protein synthesis, protease activation, ubiquitin conjugation, and autophagy (Mammucari et al. 2007, Stitt et al. 2004, Wing 2005, Zhao et al. 2007). In the past few years, there have been significant advances in elucidation of signal transduction pathways that regulate the balance between skeletal muscle protein synthesis and degradation. An increased rate of proteolysis has been identified as a major step in muscle atrophy (Krawiec et al. 2005). More significantly, the most studied proteases in skeletal muscle are lysosomal proteases, caspase-3, Ca²⁺-activated proteases (calpain) and the ubiquitin-proteasome pathway (UPP). This review will mainly focus on modulation of protein degradation by calpain and its crosstalk with UPP and Akt along with their contribution towards muscle atrophy.

Calpains
Calpains are Ca²⁺-dependent cysteine proteases that are located in all vertebrate cells (Goll et al. 2003). The calpain family is comprised of 14 members, and
muscle tissue expresses mainly three distinct calpains: the ubiquitous calpain1 and calpain2 (also called u- and m-) and calpain3 (also called p94). The typical structure of a calpain is composed of four distinct domains (Fig. 1). Calpain1 and calpain2 are heterodimers composed of two subunits of 80 and 30 kDa, respectively. The 80 kDa subunit is bond non-covalently to 30 kDa subunit which contains hydrophobic residues (domain V) and calcium binding sites (domain VI) (Fig. 1, Lin et al. 1997). The larger subunit contains the catalytic domain, whereas the smaller unit has regulatory functions. In addition to the structure elements found on calpain1 and calpain2, calpain3 possesses three unique sequences not found in other calpains including NS at N-terminus, IS1 within the catalytic domain and IS2 upstream of Ca\(^{2+}\)-binding domain. The latter two sequences confer specific properties to calpain3: IS1 possesses autolytic sites and IS2 comprises a nuclear localization signal and a binding site to titin (also called connectin), a giant elastic protein present in the sarcomere (Beckmann and Spencer 2008).

Fig. 1. (A) Four domains have been identified in both calpain1 and calpain2: (I) the N-terminal domain, (II) a domain containing a sequence characteristic of cysteine proteases, (III) a connecting domain, and (IV) a Ca\(^{2+}\)-binding domain. (B) Calpain3 carries three unique sequences: NS, IS1 and IS2.

Based on the many substrate proteins identified in vivo and in vitro, calpains are implicated in cytoskeletal remodeling via regulation of attachment of cytoskeletal proteins to plasma membrane (Mazères et al. 2006). This process has been known to be important for the physiological functions including cell fusion and cell motility. Disruption of murine calpain4 resulted inactivation of both calpain1 and calpain2, which together led to lethal phenotype in which embryos died at midgestation with defects in cardiovascular system, hemorrhaging and accumulation of erythroid progenitors (Arthur et al. 2000). These are characteristics for loss of physiological cytoskeletal remodeling essential for normal embryo development. Disruption of murine calpain3 was not lethal but the myotubes from calpain3-deficient mice lacked well-organized sarcomeres (Kramerova et al. 2004). Thus, calpain3 plays an important role in sarcomere remodeling. Indeed, loss-of-function mutations in calpain3 gene have been associated with limb-girdle muscular dystrophy type 2A and tibial muscular dystrophy (Charton et al. 2015). In addition, calpains are also important for signal transduction (Jungwirth et al. 2014), cell cycle (Liang et al. 2015), apoptosis (Nozaki et al. 2011), regulation of gene expression (Qin et al. 2010), and even for long-term potentiation which is believed to be molecular basis of memory response in neurons (Lynch 1998). Because of the wide spectrum of calpain activities, deregulation of calpain activation have been implicated in various pathological conditions including traumatic spinal cord and brain injuries, cataract formation, cerebral and heart ischemia, hypertension, arthritis, etc. (Branca 2004).

**Calpains in muscle wasting**

Several studies have established that abnormally enhanced calpain activation is commonly observed in atrophic conditions like disuse, denervation, glucocorticoid treatment and sepsis (Nelson et al. 2012, Matsumoto et al. 2014, Fareed et al. 2006). Forsberg’s team applied several strategies to downregulate intracellular calpain activities like inhibition of calpain expression by use of calpain inhibitor, overexpression of dominant-negative form of calpain2 or endogenous inhibitor calpastatin. They found that under conditions of accelerated degradation, inhibition of calpain2 reduced protein degradation by 30 %, whereas calpastatin reduced
degradation by 63%. Western blot analysis showed that cytoskeletal proteins, like fodrin and nebulin, were stabilized by inhibition of calpain2. These observations indicate that calpains play key roles in the disassembly of sarcomeric proteins (Huang and Forsberg 1998, Purintrapiban et al. 2003). In addition, the availability of transgenic mice or calpastatin-overexpressing mdx mice confirmed that calpains are involved in muscle wasting (Tidball and Spencer 2002, Spencer and Mellgren 2002). Researchers found that expression of the transgene resulted in increase of calpastatin concentration and elimination of calpain activity. Moreover, calpastatin overexpression completely prevented the shift in myofibrillar myosin content from slow to fast isoforms. Other researchers (Williams et al. 1999, Fischer et al. 2001) observed that sepsis increased expression of calpains and calcium-dependent release of myofilaments in skeletal muscle in septic rats. Either in vitro or in vivo, it has shown that null mutation of calpain3 in mice causes abnormal sarcomere formation (Kramerova et al. 2004).

Mechanisms of calpains in muscle wasting

Since proteolytic activities are potentially deleterious to the cell, calpains should be in inactivated state on most of the time (Goll et al. 2003, Taveau et al. 2003). Ca^{2+} spikes, removal of N-terminus region via autolysis, phosphorylation, membrane association and calpastatin all seem to be involved in the regulation of calpain activities (Goll et al. 2003). Among them, calcium and endogenous inhibitor calpastatin are the two major regulators on calpains activation. Calpain1 and calpain2 have different in vitro calcium sensibility with range of 5-50 μM for calpain1 and 250-1000 μM for calpain2 (Elce et al. 1997). It is clear that skeletal muscle atrophy induced by calpains is associated with an increase in cytosolic calcium levels (Kourie 1998). For example, intracellular concentrations of calcium are increased in skeletal muscle during sepsis (Fischer et al. 2001).

Calpains exists in the cytosol as an inactive form and translocates to membranes in response to increases in intracellular Ca^{2+} level. At the membrane, calpain is activated in the presence of Ca^{2+} and phospholipids. Process of calpains activation by Ca^{2+} concludes two steps. First, domain interaction imposes release of structural constraints which then leads to dissociation of 30 kDa from 80 kDa. Second, rearrangement of the active cleft is caused by binding of two Ca^{2+} atoms to the protease domain. In the cases of calpains without 30 kDa, the first step of activation might be bypassed and they are activated directly by the second stage. Ca^{2+}-induced structural changes that release the constraints are prerequisite for activation to form a functional catalytic site. Activated calpain or 80 kDa hydrolyzes substrate proteins at membranes or in cytosol after release from membranes. Phosphorylation of calpain might be another important mechanism for activity regulation. Phosphorylation of calpain at Ser369 in domain III by protein kinase A restricts domain movement and freezes calpain2 in an inactive state (Shiraha et al. 2002). Of note, anchoring to titin keeps calpain3 from autolytically degrading itself and maintains it in a proteolytically inactive state. Robyn et al. (2006) stated that calpain3 is bound tightly within a fiber, whereas most calpain1 is initially freely diffusible in the cytoplasm at resting [Ca^{2+}] under physiological conditions. These findings demonstrated that the process is precisely attuned to avoid uncontrolled proteolytic activity under normal circumstances. Moreover, these findings indicated that substantial proteolytic damage may be resulted if resting or localized calcium is elevated, which is likely to occur during eccentric contraction and in dystrophic muscle.

As we know, skeletal muscle contains 3 groups of proteins: myofibrillar proteins, sarcoplasmic proteins, stroma proteins. The myofibrillar proteins are not only the largest class of skeletal muscle proteins but also are responsible for the contractile properties of muscle. The contractile function of myofibrils requires the myofibrillar structure extend continuously from one end of the muscle cell to the other. Thus, turnover of myofibrillar proteins must be accomplished without disrupting this continuous structure. This mechanism is consistent with the observations that atrophying muscle in different metabolic conditions has smaller diameter myofibrils than unaffected muscle (Badalamente and Stracher 2000).

Myofibrils are composed of functional mixtures of proteins which include sarcomeric (i.e. contractile) and cytoskeletal proteins, and the latter actually account for nearly 50% of adult protein mass. Calpains residing within sarcomere are also associated with formation of sarcolemma mainly through controlling of early events of sarcomeric protein disassembly. Cytoskeletal proteins like desmin, vimentin, dystrophin, filamin and sarcomeric proteins have all been reported to be substrates of calpains in vivo or in vitro (Table 1). Due to the strong cleavage activities on these critical cytoskeletal proteins demonstrated by calpains, Dayton et al. (1976a, b) proposed that the calpains might be responsible for release of myofilaments from the surface of myofibrils.
Table 1. Some cytoskeletal proteins known as substrates of calpains (Goll et al. 2003).

<table>
<thead>
<tr>
<th>Polypeptide name</th>
<th>Effects of calpain cleavage</th>
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<tbody>
<tr>
<td><strong>Adducin</strong></td>
<td>Both the 103-kDa-subunit and the 97-kDa-subunit are degraded to produce stable 57- and 49-kDa fragments, respectively; the 49-kDa-subunit fragment does not bind calmodulin as its parent97-kDa does (Scaramuzzino and Morrow 1993).</td>
</tr>
<tr>
<td><strong>Ankyrin</strong></td>
<td>Both the brain (212 kDa) and erythrocyte (239 kDa) isoforms are degraded to a 160-kDa polypeptide (Harada et al. 1997).</td>
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<tr>
<td><strong>Caldesmon</strong></td>
<td>Degraded to polypeptide fragments of 125-, 115-, 100-, 105-, and 88-90 kDa; the latter fragment is stable (Croall et al. 1996).</td>
</tr>
<tr>
<td><strong>Cadherin</strong></td>
<td>E-cadherin is not degraded by calpain (Bush et al. 2000), whereas the intracellular domain of N-cadherin is degraded by calpain1 (Covault et al. 1991) or calpain2 (Sato et al. 1995).</td>
</tr>
<tr>
<td><strong>Calponin</strong></td>
<td>Degraded to 30-, 27-, and 19.5-kDa fragments; the latter fragment is stable (Croall et al. 1996, Tsunekawa et al. 1989).</td>
</tr>
<tr>
<td><strong>Catenin</strong></td>
<td>α-catenin is not degraded by calpain but calpain removes the NH₂ terminus of β-catenin to produce 90- and 75-kDa fragments from the 97-kDa β-catenin.</td>
</tr>
<tr>
<td><strong>C-protein</strong></td>
<td>Small fragment removed from the native 140-kDa polypeptide leaving a 120-kDa fragment (SDS-PAGE); this degradation can occur while the C-protein is bound to the myofibril (Dayton et al. 1975).</td>
</tr>
<tr>
<td><strong>Desmin</strong></td>
<td>Degraded to a 32- to 37-kDa stable fragment; an 18-kDa fragment appears after longer digestion (O'Shea et al. 1979); a 9-kDa fragment is removed from the NH₂ terminus leaving the central rod domain; calpain degradation destroys the ability of desmin to self-assemble and to bind nucleic acids (Nelson and Traub 1983).</td>
</tr>
<tr>
<td><strong>Dystrophin</strong></td>
<td>Degraded to a 30-kDa NH₂-terminal fragment and several 50- to 140-kDa fragments (Yoshida et al. 1992).</td>
</tr>
<tr>
<td><strong>Gelsolin</strong></td>
<td>Degraded to a 40-kDa fragment that contains the actin-binding segment of gelsolin and a 45-kDa fragment that contains the Ca²⁺-binding properties of gelsolin.</td>
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<tr>
<td><strong>Filamin/actin-binding protein</strong></td>
<td>Degraded to 240- and 10-kDa fragments that no longer have the cross-linking abilities of the undegraded filamin (Davies et al. 1978).</td>
</tr>
<tr>
<td><strong>Myosin</strong></td>
<td>Degradation of undenatured myosin; the 210-kDa large subunit is degraded to fragments of 150-, 165-, and 180-kDa; degradation of LC2 light chain (Pennick and Grebenau 1984).</td>
</tr>
<tr>
<td><strong>Nebulin</strong></td>
<td>Degraded to a series of smaller polypeptides ranging from 30 to several hundred kDa; fragments produced by calpain may remain bound to actin (Taylor et al. 1995); calpain degradation severs nebulin connection to Z-disc.</td>
</tr>
<tr>
<td><strong>αII-spectrin</strong></td>
<td>Degraded to 145- and 150-kDa fragments; the 150-kDa fragment may be detected by a specific antibody; the 145- and 150-kDa fragments are then degraded to smaller fragments (Yoshida et al. 1995); the initial calpain sensitive site is at Tyr₁₁⁷⁶-Gly₁₁⁷⁷ (Stabach et al. 1997).</td>
</tr>
<tr>
<td><strong>Talin</strong></td>
<td>Cleaved between Q₂₃ and Q₂₃₄ (chicken talin) to produce a 190-kDa COOH-terminal actin binding fragment and a 47-kDa NH₂-terminal fragment; after cleavage, can no longer cross-link integrin to cytoskeletal elements (Hemmings et al. 1996, Muguruma et al. 1995).</td>
</tr>
<tr>
<td><strong>Titin</strong></td>
<td>The ~3000-kDa titin polypeptide is cleaved to a large ~2000-kDa fragment by removal of a 1200-kDa NH₂-terminal fragment; this cleavage severs the connection of titin to the Z-disc; the 1200-kDa fragment is degraded to smaller fragments of 100-500 kDa, with the 500-kDa fragment being stable (Taylor et al. 1995); chicken titin is quickly degraded to a T2 (2000 kDa) fragment which is then degraded to a 1700- and 300-kDa fragment; the 1700-kDa fragment is further degraded to a 1400-kDa fragment (Suzuki et al. 1996).</td>
</tr>
<tr>
<td><strong>Tropomyosin</strong></td>
<td>Cleaved to a 14-kDa fragment and several smaller polypeptides (Dayton et al. 1975).</td>
</tr>
<tr>
<td><strong>Troponin I</strong></td>
<td>Degraded to smaller polypeptides (the 32-kDa cardiac troponin I is degraded to a 26-kDa fragment as measured by SDS-PAGE) at a moderate rate; not protected while in the myofibrillar structure (Dayton et al. 1975).</td>
</tr>
<tr>
<td><strong>Troponin T</strong></td>
<td>Degraded to smaller fragments of 35-, 30-, and 28-kDa as measured by SDS-PAGE; the 30-kDa fragment is stable; a 15-kDa fragment is produced by longer digestion (Ho et al. 1994); not protected when in the myofibrillar structure (Dayton et al. 1975).</td>
</tr>
<tr>
<td><strong>Tubulin</strong></td>
<td>Degraded from a 55- to 50- to 52-kDa fragment (Billger et al. 1988); or not degraded at all (Sandoval and Weber 1978).</td>
</tr>
<tr>
<td><strong>Vinculin</strong></td>
<td>Cleaved to a ~90-kDa fragment (Taylor et al. 1995) that can no longer cross-link talin, paxillin, and α-actinin filaments.</td>
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**UPP pathways**

The essential feature of the UPP is that proteins are tagged with a polyubiquitin chain, which marks them for degradation by the 26S proteasome, a tubular multisubunit complex containing proteolytic enzymes on the luminal side of the proteasome chamber. The whole proteasome complex (26S) is comprised of a core proteasome subunit (20S) that is coupled with a regulatory complex (19S) at both ends (Hasselgren 1999, Tidball and Spencer 2002, Grune and Davies 2003, Grune et al. 2003). Interestingly, proteins can be degraded by either the 26S proteasome or the 20S protease core. 26S proteasome degradation pathway is only active machinery when protein substrates are ubiquitinated and marked for degradation. Addition of ubiquitin to a protein substrate is believed to be an exquisitely modulated process. This process requires three distinct components: an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme and an E3 ubiquitin ligating enzyme (Hershko and Ciechanover 1998, Cohen et al. 2009). UPP-mediated protein degradation is a selective process with two distinct and successive steps which include covalent attachment of multiple ubiquitin molecules to the target protein substrates and subsequent degradation of the tagged substrates by 26S-proteasome (Schwartz and Ciechanover 2009). Conjugation of ubiquitin to the substrate proceeds via a three-step enzymatic mechanism with E3 ubiquitin ligases being the rate-limiting step (Ciechanover et al. 2000). Ubiquitin is first activated by ubiquitin activating enzyme (E1). This activated ubiquitin is then transferred to E2. E3 transfers an activated form of ubiquitin from E2 to the lysine residue on the substrate. The E3 enzymes are the primary determinant of substrate specificity because
they are able to recognize several structural motifs like the amino terminal residue of the substrate, specific phosphorylated domains or the “destruction box” (Lecker et al. 1999a). Individual E3 ubiquitinate specific classes of proteins; hence the E3s play an important role in determining which proteins are targeted for degradation by the proteasome.

Muscle atrophy induced by UPP

It has been shown that degradation through the UPP may account for up to 80 % of proteolysis during skeletal muscle wasting (Tawa et al. 1997). Studies of experimental animal models and patients have consistently demonstrated that the UPP is responsible for degradation of muscle proteins in bulk volume. More importantly, UPP becomes activated in catabolic states associated with atrophy (Sandri 2013, Smuder et al. 2014). In most atrophy models studied, levels of UPP components transcripts are increased, and higher rates of ubiquitin conjugation were found in vitro (Jagoe et al. 2002, Lecker et al. 2004, 1999b, Stevenson et al. 2003). Studies of differentiated myotube cultures demonstrated that treatment of myotubes with cachaetic glucocorticoid dexamethasone increased expression of genes broadly involved in UPP proteolytic pathway correlating well with enhanced protein breakdown (Du et al. 2000, Hong and Forsberg 1995, Wang et al. 1998). The unique ubiquitin E3 ligases, muscle atrophy F-box (MAFbx) and muscle ring finger-1 (MuRF-1) exist in skeletal muscle, and these ligases play essential roles in skeletal muscle atrophy (Foletta et al. 2011). In vitro treatment of myotubes with DEX induces atrophy was accompanied with specifically increased expression of MAFbx and MuRF1 (Sandri et al. 2004, Stitt et al. 2004), indicating MAFbx and MuRF1 are negatively associated with presence of muscle atrophy. Indeed, mice lacking MAFbx or MuRF1 genes were found to be resistant to atrophy (Bodine et al. 2001a, Furlow et al. 2013). For example, both MAFbx and MuRF1 knockout mice showed a significant attenuation in loss of muscle mass after denervation comparing to wild-type mice.

Relationship between calpains and UPP

Proteasome is able to degrade only sarcomeric proteins in monomeric form (including α-actin) but not when they are in higher-ordered structures. Higher-ordered proteins are much more stable when being associated with each other in the actomyosin complex (accounted for 50-70 % of muscle proteins) or intact myofibrils (Tidball and Spencer 2002). On one hand, the size of proteasome is so large that it is not possible for them to enter the sarcomere and to digest their proteins. On the other hand, the entrance to the central cavity of the proteasome containing the active sites is only 10-13 Å in diameter which is much narrower for entry of myofilaments ranging from 10 to 100 μm in diameter. Thus, the first step in degradation of myofibrillar proteins (actin and myosin) during atrophy requires release of myofilaments from the sarcomere (Solomon and Goldberg 1996, Solomon et al. 1998). These initial steps in myofibrillar proteolysis appear to rely on the calpain activation and caspase systems.

Overall, loss of myofibrillar proteins in muscle wasting requires the concerted action of at least two proteolytic systems. Calpains can be regarded as the initiators of myofibrillar degradation. As previously stated, several proteins important for the structural integrity of the sarcomere such as nebulin, titin and filamin are readily cleaved by calpain (Huang and Forsberg 1998, Smith et al. 2008). The consequences are deattachment of these structural proteins in the Z-disc and release of principal Z-disc proteins like α-actinin. Thus, calpains rapidly cleave sarcomeric proteins and result in filament release from the myofibril. Once filament released, UPP plays an essential role in their proteolysis. E3 ubiquitin ligases, MuRF1 and MAFbx are essential for UPS-mediated proteolysis of filament proteins (Clarke et al. 2007, Cohen et al. 2009). MuRF1 and MAFbx mediate atrophy by ubiquitinating particular protein substrates that further degraded by proteasome. MuRF1’s substrates include several components of the sarcomeric thick filament, e.g. myosin heavy chain (Clarke et al. 2007). It also has been showed that MuRF1 binds to myofibrillar protein titin at the M line (Centner et al. 2001, McEllhinney et al. 2002, Pizon et al. 2002). Subsequently, it was shown that several other proteins in the thick filament of muscle were also degraded by MuRF1, including myosin light chain and myosin binding protein C (Cohen et al. 2009). Smith and Dodd (2007) designed ex vivo experiments using isolated rat diaphragm muscle treated with Ca2+, calpain inhibitor calpeptin or proteasome inhibitor epoxomicin. They found that calpain activation increased total protein degradation and proteasome-dependent proteolysis by 65 % and 144 %, respectively. In addition, when proteasomes were inhibited, the increase in proteolysis following calpain activation was ameliorated. Hence, calpain and UPP could have synergistic reaction on
Akt signaling pathway

The serine/threonine kinase Akt, also known as protein kinase B, is a central node in cell signaling downstream of growth factors, cytokines, and other cellular stimuli. As a transducer it stimulates signaling to its downstream cascades through phosphorylation of a myriad of substrates, resulting in the integration of anabolic, catabolic and mechanical responses (Clemmons 2009, Wu et al. 2010). Two major downstream pathways of Akt relevant to muscle hypertrophy are the mammalian target of rapamycin (mTOR) and glycogen synthase kinase 3β (GSK3β). Both contribute to control of protein synthesis. A third downstream pathway of Akt is the Forkhead Box O (FoxO) transcription factor which controls protein degradation.

Akt and muscle hypertrophy

Evidences both in rats and humans have shown that Akt activity is increased in response to muscle contraction and hormonal or growth factor stimulation (Nader and Esser 2001, Sakamoto et al. 2002, 2003). For example, insulin or IGF1 can phosphorylate and activate Akt both in vitro (Takahashi et al. 2002) and in vivo (Bodine et al. 2001b, Pallafacchina et al. 2002). Furthermore, presence of a constitutively active form of Akt in skeletal muscle cells is able to result muscle hypertrophy probably via abnormal activation of its downstream pathways. Phosphorylated Akt subsequently phosphorylates and activates mTOR which then activates p70S6K and increases inhibitory effect of PHAS-1 to eIF4E (Terada et al. 1994). All these can promote protein synthesis (Stitt et al. 2004, Glass 2005, Rena et al. 1999). Meanwhile, Akt activation reduces GSK-3β kinase activity through phosphorylation of Ser9 on GSK-3β (Harwood 2001). Phosphorylated GSK-3β is able to enhance activities of eIF2B (eukaryotic initiation factor 2B) resulting increased mRNA translation (Welsh et al. 1998). Therefore, the IGF-1/Akt/mTOR and IGF-1/Akt/GSK-3β pathways are playing mediating effects for muscle hypertrophy caused by dysregulated Akt.

Akt and muscle atrophy

In vast majority of atrophy conditions, especially in rodents, the reduction of Akt activation is associated with activation in the FoxO/MAFbx pathway. The reduced activity of Akt found in various models of muscle atrophy lead to decreased phosphorylation of FoxO which hence results its accumulation in the nucleus since phosphorylated FoxO by Akt will export out of nucleus (Calnan and Brunet 2008). The translocation and transcriptional activation of FoxO members is sufficient to promote both a MAFbx and MuRF1 expression (Bodine et al. 2001a, Gomes et al. 2001). Moreover, studies revealed that TNF-α activation of FoxO transcription of MAFbx was paralleled by an increase in Akt activity (Stitt et al. 2004). Léger et al. (2006) found that Akt was reduced while MAFbx mRNA and protein levels were increased in amyotrophic lateral sclerosis (ALS) patients and ALS G93A mice. This occurred without a change in nuclear FoxO levels, suggesting that MAFbx transcription may be regulated by another Akt stimulated pathway.

Research by Sugita et al. (2005) found that burn injury impairs phosphorylation of Akt and activation of GSK-3β in skeletal muscle. These findings suggested that attenuated Akt activation was involved in disturbed metabolism and muscle wasting. Other research by Fang et al. (2007) reported that burn injury increased GSK-3β kinase activity in atrophying muscle and significantly lowered Akt kinase activity, which was further confirmed by reduced levels of phosphorylated Akt. In addition, hindlimb unloading induced by suspending rats for 14 days, results in muscle atrophy with a decrease in total and phosphorylated Akt, reduction of phosphorylation of p70S6K and increase in binding of PHAS-1 to eIF4E (Bodine et al. 2001b). These indicate that interfering with other pathways of Akt, mTOR or GSK-3β, may also regulate muscle atrophy.

Relationship between calpains and Akt

As mentioned previously, calpain mainly destructs skeleton structural proteins whereas Akt mainly perturbs translation or transcription of mRNA of skeleton proteins. Studies by Sato et al. (2000) revealed that molecular chaperone heat shock protein90 (HSP90) can bind and protect phosphorylated Akt from dephosphorylation by phosphatase2A to maintain Akt activation. In addition, they also demonstrated that HSP90 is a calpain substrate in which calpain activation reduces HSP90-client proteins binding (Stalker et al. 2003). These suggest that calpain activation may diminish HSP90-Akt binding in skeletal muscle thus reducing Akt activation. A study by Smith and Dodd (2007) demonstrated that presence of Ca²⁺ significantly decreased HSP90 content by 33% which can be
prevented by inhibition of calpain activation. Therefore, increased calpain-dependent protein degradation seen in calcium-treated muscles could be associated with reduced HSP90 and hence reduced Akt activation. Moreover, reduction of Akt activities will allow nuclear translocation and activation of FoxO transcription factors which are vital in regulating the expression and activity of MAFbx and MuRF1, thereby initiation of UPP-mediated muscle proteolysis (Sandri et al. 2004, Stitt et al. 2004, Glass 2005).

Association of calpains with other signaling pathway

Reports indicated that oxidative stress is capable of promoting calpain expression in muscle cells in culture. Exposure of C2C12 myotubes and human myoblasts to H2O2 resulted an increase in expression of calpains (McClung et al. 2009, Dargelos et al. 2010). Studies have reported that intracellular production of reactive oxygen species (ROS) could play a role in disturbances of calcium homeostasis (Kandarian and Stevenson 2002). A potential mechanism to link oxidative stress with calcium overload is that ROS-mediated formation of reactive aldehydes which is known to be able to inhibit plasma membrane Ca2+-ATPase activity (Siems et al. 2003). Therefore, an oxidative stress-induced decrease in membrane Ca2+-ATPase activity would impede Ca2+ removal from the cell and promote intracellular Ca2+ accumulation and calpains activation.

Calpain activation may also regulate degradation of various transcription factors involved in muscle wasting which include signal transducer, transcription family (STAT) and nuclear factor-kB (Oda et al. 2002, Wei et al. 2006). In addition, intracellular signaling molecules, like protein kinase C, calcineurin and Cdc42/RhoA, were also cleaved by calpain to regulate muscle atrophies (Chockalingam et al. 2002, Sneddon et al. 2000, Wang et al. 1989). Therefore, we predict that new concepts concerning calpain regulation of muscle wasting will soon emerge.

Clinical implications

Skeletal muscle atrophy is a major clinical problem, as it occurs in a large group of patients, such as sepsis, neuromuscular disorders and prolonged mechanical ventilation (MV). It increases the risk of complications and puts a huge financial burden on the healthcare system. No therapies are currently available that improve skeletal muscle weakness. A recent data on the effects of MV on the human diaphragm indicates that diaphragm muscle fibers display atrophy, contractile weakness, and activation of UPP (Pleuni et al. 2015). However, studies showed that absence effect of inhibition of UPP on MV-induced muscle atrophy (Smuder et al. 2014, Agten et al. 2012). Meanwhile, inhibition of the calpain activity preserves sarcomeric structure, prevents the development of muscle weakness and muscle atrophy both in peripheral muscle and diaphragm (Nelson et al. 2012, Salazar et al. 2010). Therefore, to investigate the molecular mechanisms of calpains on muscle atrophy is of clinical importance in hope to stimulate the interest in development of novel therapeutic interventions.

Conflict of Interest

There is no conflict of interest.

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