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The International Journal of Biochemistry & Cell Biology 37 (2005) 1985–1996
IJBCB
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Review

Molecular determinants of skeletal muscle mass: getting the "AKT" together

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Received 16 November 2004; received in revised form 1 February 2005; accepted 22 February 2005

Abstract

Skeletal muscle is the most abundant tissue in the human body and its normal physiology plays a fundamental role in health and disease. During many disease states, a dramatic loss of skeletal muscle mass (atrophy) is observed. In contrast, physical exercise is capable of producing significant increases in muscle mass (hypertrophy). Maintenance of skeletal muscle mass is often viewed as the net result of the balance between two separate processes, namely protein synthesis and protein degradation. However, these two biochemical processes are not occurring independent of each other but they rather appear to be finely coordinated by a web of intricate signaling networks. Such signaling networks are in charge of executing environmental and cellular cues that will ultimate determine whether muscle proteins are synthesized or degraded. In this review, recent findings are discussed demonstrating that the AKT1/FOXOs/Atrogin-1(MAFbx)/MuRF1 signaling network plays an important role in the progression of skeletal muscle atrophy. These novel findings highlight an important mechanism that coordinates the activation of the protein synthesis machinery with the activation of a genetic program responsible for the degradation of muscle proteins during skeletal muscle atrophy.

Keywords: Hypertrophy; Atrophy; Intracellular signaling; Exercise; AKT/PKB; FOXO

Contents

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doi:10.1016/j.biocel.2005.02.026

1. Introduction

Skeletal muscle is the most abundant tissue in the human body accounting for ∼50% of the total body mass. It is not only the major site of metabolic activity but it is also the largest protein reservoir, serving as a source of amino acids to be utilized for energy production during periods of food deprivation, and playing a central role in nitrogen flow during some disease states. Over the years, a large body of evidence has suggested that in many disease states or unfavorable environmental conditions, skeletal muscle mass could be markedly reduced, a condition that may have devastating health consequences. In contrast, some forms of physical activity such as resistance exercise, can produce large increases in skeletal muscle mass. Clearly these two contrasting situations represent both ends of a continuum of mechanisms involved in balancing the forces that regulate skeletal muscle mass. Understanding such mechanisms could lead to a better management of the loss of skeletal muscle.

Two recent studies have further expanded our knowledge about the mechanisms involved in the development of skeletal muscle atrophy. In these reports, Sandri et al. (2004) and Stitt et al. (2004) together with their respective co-workers have provided direct evidence for a role of AKT1 signaling as a modulator of the expression of two important genes involved in the progression of muscle atrophy, the E3 ubiquitin ligases atrogin-1 or Muscle Atrophy F box (MAFbx) and the Muscle Ring Finger-1 (MuRF-1), and their regulation by a family of transcription factors termed Forkhead box O (FOXO). Therefore the specific goal of the present review is to discuss the role of AKT1 as a regulator of the expression of these atrophy-related genes via the FOXO family of transcription factors (FOXOs) and the integration of such mechanism in a signaling network previously characterized involving AKT1 signaling in the activation of the protein synthetic machinery. Exciting new evidence demonstrates that the expression of atrogin-1 (MAFbx) and MURF-1 is controlled by a signaling network that comprises FOXOs and their regulation by AKT1. These new findings are important not only from the atrophy standpoint, but also from the integration of cellular regulatory networks perspective as they created a scenario in which a key molecule that is positively involved in cellular growth (via protein synthesis) when in its active state, also negatively regulates the opposite process (protein degradation). Such interaction suggests that the dynamic regulation of skeletal muscle mass is not simply the balance between protein synthesis and degradation but is a rather finely coordinate process.

2. Signaling networks regulating skeletal muscle mass

The regulation of skeletal muscle mass is a rather complex phenomenon, and several excellent reviews have been recently published addressing this topic in great detail (Glass, 2003; Jackman & Kandarian, 2004; Lecker, Solomon, Mitch, & Goldberg, 1999; Rennie, Wackerhage, Spangenburg, & Booth, 2004; Sartorelli & Fulco, 2004). In general, muscle hypertrophy is the result of an increase in the size of the existing muscle fibers. Such increase is reflected by the increase in cross-sectional area of the muscle fibers, which in turn is a consequence of the accumulation of contractile proteins within the fiber. In stark contrast, muscle atrophy (also called muscle wasting) is a consequence of the loss of such contractile proteins due to a reduction in muscle fiber cross-sectional area. In essence, the maintenance of skeletal muscle mass is the result of the dynamic balance between muscle protein synthesis and muscle protein degradation. Therefore, these two opposite processes are believed to hold the key to the understanding of the mechanisms involved in the regulation of skeletal muscle mass.

2.1. Mechanisms of muscle protein synthesis and protein degradation: effects of resistance exercise versus inactivity and disease states

Several recent studies have pointed towards a unique series of signaling mechanisms associated with exercise-induced skeletal muscle hypertrophy. One such target of this signaling network important for skeletal muscle hypertrophy is the activation of the protein synthetic machinery (Bolster, Kimball, & Jefferson, 2003; Nader, Hornberger, & Esser, 2002; Rennie et al., 2004) (Fig. 1). Initial reports have demonstrated that an acute bout of a resistance exercise paradigm known to result in skeletal muscle hypertrophy was capable of inducing a sustained increase in protein synthesis rates for at least 24 h following the exercise bout (Hernandez, Fedele, & Farrell, 2000). This increase in protein synthesis was correlated with the activation of the phosphoinositide-3 kinase (PI3K), the mammalian target of rapamycin (mTOR) and the 70 kDa ribosomal S6 protein kinase (S6K1/p70^{S6k}) (Baar & Esser, 1999; Bolster, Kubica et al., 2003; Hernandez et al., 2000). Indeed, such modality of exercise also resulted in the activation of AKT (Bolster, Kubica et al., 2003; Nader & Esser, 2001), however when compared with other types of contractile activity such as endurance exercise, the activation of AKT and S6K1 was specific to the resistance exercise stimulus (Bolster, Kubica et al., 2003; Nader & Esser, 2001), suggesting that activation of the PI3K/AKT/mTOR/S6K1 signaling network was associated with the growth response of skeletal muscle to resistance exercise. A more definite assessment of the signaling mechanisms involved in exercise-induced skeletal muscle hypertrophy was provided by studies utilizing rapamycin, a specific mTOR inhibitor. Bodine, Stitt et al. (2001) have subjected the plantaris muscles of rats to functional overload by removing the synergist muscles (soleus and gastrocnemius). After 14 days of overload, the plantaris muscles were ∼45% larger than control. Administration of rapamycin resulted in a complete abrogation of this response, indicating that signaling through mTOR is essential for skeletal muscle growth.

Opposite to the changes in protein synthesis, degradation of cellular proteins is an essential process for the maintenance of cellular homeostasis. Proper cellu-

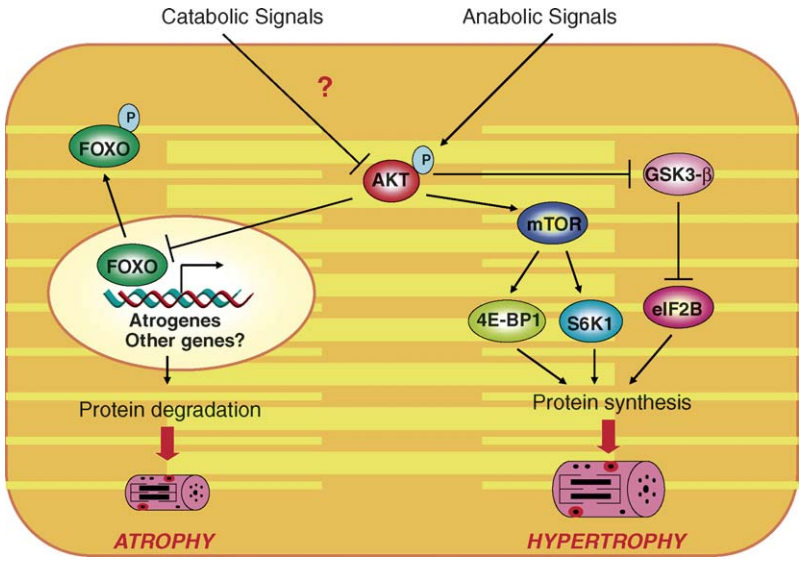

Fig. 1. AKT signaling network during hypertrophy and atrophy. AKT lies at the center of a network in which its activation regulates the protein synthetic machinery and its inactivation allows the expression of atrogin-1(MAFbx), MuRF-1 and possibly other atrophy-associated genes (atrogenes). Stimulation of skeletal muscle with anabolic agents such as IGF-1 leads to the activation of AKT1 and activation of the protein synthetic machinery via mTOR and S6K1 while inactivating the protein synthesis repressors 4E-BP1 and GSK3-β. Conversely, catabolic agents such as glucocorticoids result in the inactivation of AKT and this leads to the dephosphorylation of FOXOs, their nuclear translocation and subsequent regulation of atrogenes (→ activation, —| inhibition).

lar function requires an adequate quality control so as to ensure the degradation of proteins involved in regulatory processes, the renewal of structural and damaged proteins, and to prevent of the accumulation of misfolded proteins (Goldberg, 2003). However, in some specific situations when protein degradation exceeds protein synthesis, skeletal muscle wasting occurs. This loss of muscle mass is a hallmark of conditions such as exposure to microgravity (Adams, Caiozzo, & Baldwin, 2003), aging (Singh, 2002), renal disease (Mitch & Price, 2001), cancer (Baracos, 2001), critical illness (Di Giovanni et al., 2004), diabetes (Price et al., 1996) and HIV-AIDS (Miro et al., 1997) among others. The lack of proper interventions that could ameliorate the loss of muscle mass during these conditions represents a major impediment for the proper management of some of these diseases and remains as a high morbidity factor.

Over the years, several studies have identified at least four different systems involved in the degradation of proteins during muscle atrophy. These are the lysosomal system (Voisin et al., 1996), the calpain system (Huang & Forsberg, 1998), the caspase or apoptotic protease system (Du et al., 2004; Lee et al., 2004), and the ubiquitin proteasome system (Lecker et al., 1999). At present, it remains unclear what the relative contribution of these systems to the atrophy process are, and which specific roles they may play during each particular disease state or context in which muscle atrophy develops, i.e. sepsis versus sarcopenia (Jackman & Kandarian, 2004). Among the various systems involved in muscle protein degradation, the ubiquitin proteasome system is thought to play a major role in skeletal muscle atrophy (Lecker et al., 1999), and has recently experienced a renewed interest due to the identification of two new members of the family of enzymes involved in selecting proteins for their degradation, the ubiquitin E3 ligases atrogin-1(MAFbx) and MuRF-1 (Bodine, Latres et al., 2001; Gomes, Lecker, Jagoe, Navon, & Goldberg, 2001). Both ligases have been identified to be specifically expressed in skeletal muscle. Gomes et al. (2001) have identified atrogin-1 as a gene that was upregulated 7–9-fold in muscles from food-deprived mice and subsequently found that atrogin-1 expression was also elevated in muscles from animals that experienced muscle atrophy due to diabetes, cancer and renal failure. In another study, Bodine, Latres et al. (2001) have identified the same gene which they termed MAFbx and another gene, named MuRF-1. Both MAFbx and Murf-1 were found to be specifically expressed and upregulated in skeletal muscles of rats undergoing atrophy induced by immobilization, denervation and hindlimb suspension.

3. Intracellular signaling mechanisms involved in the regulation of skeletal muscle mass: role of AKT

As mentioned above, the increase in skeletal muscle mass is, in part, a consequence of an increase in protein accumulation due to increases in protein synthesis rates (Bolster, Kimball et al., 2003; Nader et al., 2002; Rennie et al., 2004). Protein synthesis is regulated at many levels and involves several intracellular signaling mechanisms (Bolster, Kimball et al., 2003; Kimball, Farrell, & Jefferson, 2002; Nader et al., 2002; Proud & Denton, 1997; Rennie et al., 2004). Among the intracellular mechanisms controlling protein synthesis, signaling via AKT1 appears to play a fundamental role in this process (Glass, 2003).

The serine-threonine kinase AKT family (also known as protein kinase B, PKB) is composed of three members: AKT1 (PKB-α), AKT2 (PKB-β) and AKT3 (PKB-γ). These three isoforms share >80% homology and they are expressed in a tissue specific manner: AKT1 and AKT2 isoforms are predominantly expressed in skeletal muscle, thymus, brain, heart and lung, and expression of the AKT3 isoform predominates in the brain and testes (Coffer & Woodgett, 1991; Jones, Jakubowicz, & Hemmings, 1991; Jones, Jakubowicz, Pitossi, Maurer, & Hemmings, 1991). Phosphorylation and activation of AKT is known to occur, for example, in response to insulin, IGF-1 and other ligands of the receptor tyrosine kinase type suggesting an important role of AKT in mediating mitogen-induced cellular functions (Alessi et al., 1996). After receptor binding, such ligands are known to recruit the activity of phosphoinositide-3 kinase (PI-3K) which in turn leads to the formation of phosphatidyl-inositol phosphates (PIPs). AKT is then targeted to the plasma membrane and becomes phosphorylated at Thr^{308} by phosphoinositide-dependent kinase (PDK)-1 (Alessi et al., 1997). Phosphorylation of another site in AKT, Ser^{473}, is believed to be the target of the integrin-linked kinase (ILK) or a yet unknown putative kinase hypo-

thetically named PDK-2 (Hannigan et al., 1996). Both phosphorylation sites in AKT are required for full kinase activity (Alessi et al., 1996, 1997; Anderson, Coadwell, Stephens, & Hawkins, 1998; Andjelkovic et al., 1997; Hannigan et al., 1996; Walker et al., 1998).

It remains to be determined, however, what role the different AKT isoforms may play in the cell. Recent genetic approaches have begun to elucidate the specific functions of the different AKT isoforms. AKT1$^{-/-}$ mice are viable but they display a somatic growth-deficient phenotype suggesting that this isoform may be directly involved in cellular growth control (Cho, Thorvaldsen, Chu, Feng, & Birnbaum, 2001). Accordingly, overexpression of an active form of AKT1 results in a hypertrophic phenotype as judged by an increase in organ size (Matsui et al., 2002; Shioi et al., 2002). In skeletal muscle specifically, expression of a constitutive active form of AKT1 results in both in vitro and in vivo muscle hypertrophy, and prevents atrophy of denervated muscles (Bodine, Stitt et al., 2001). A distinct phenotype, however, is observed in the AKT2$^{-/-}$ mice, these mice are also viable but instead develop a phenotype that resembles type II diabetes, implicating this isoform in the regulation of glucose metabolism (Cho, Mu et al., 2001). These results suggest that the different AKT isoforms may not have redundant roles but they are associated with specific cellular functions.

An intriguing aspect of AKT signaling is the wide range of cellular functions that this kinase mediates, which range from the modulation of glycolysis through the regulation of phosphofructokinase (Deprez, Vertommen, Alessi, Hue, & Rider, 1997), to the regulation of protein synthesis: AKT activity leads to the activation of mTOR and p70^{S6k}, inhibition of the cap-binding protein 4E-BP1 (Dufner, Andjelkovic, Burgering, Hemmings, & Thomas, 1999; Nave, Ouwens, Withers, Alessi, & Shepherd, 1999), and inhibition of glycogen synthase kinase 3-β (GSK3-β) (Cross, Alessi, Cohen, Andjelkovich, & Hemmings, 1995), which acts as a repressor of the eukaryotic initiation factor 2B (eIF-2B) by decreasing its capacity for ribosome recycling and thereby reducing protein synthesis at the initiation step (Jefferson, Fabian, & Kimball, 1999).

Another important function of AKT is the regulation of gene transcription through inactivation of FOXOs (Birkenkamp & Coffer, 2003; Rena, Guo, Cichy, Unterman, & Cohen, 1999) (Fig. 2).

The FOXO sub-family of transcription factors belongs to a group of evolutionarily conserved transcription factors of ∼90 members (Birkenkamp & Coffer, 2003). Three mammalian isoforms have so far been identified and relatively well characterized: FOXO-1, FOXO-3a and FOXO-4 (Biggs, Cavenee, & Arden, 2001; Biggs, Meisenhelder, Hunter, Cavenee, & Arden, 1999). (*Note*: More recently, a fourth member, FOXO-6, has been identified. This isoform will not be discussed further as it has been shown to have a different mode of regulation, and is not relevant for the purpose of the present review.) (Jacobs et al., 2003) FOXOs are predominantly located in the nuclear compartment where they are active, this means unphosphorylated and DNA bound. Phosphorylation and inactivation of FOXOs is carried out in part by AKT on at least two conserved residues (see Fig. 2 inset). This results in their release from DNA and further binding to 14-3-3 proteins. Phosphorylated FOXOs bound to 14-3-3 are then transported to the cytoplasmic compartment where they remain sequestered by 14-3-3 proteins, phosphorylated and transcriptionally inactive, and are therefore prevented from nuclear import (Birkenkamp & Coffer, 2003a). Conversely, upon inactivation of AKT, FOXOs become dephosphorylated and both FOXOs and 14-3-3 are imported back to the nucleus where FOXOs can exert their transcriptional activity (Fig. 2). FOXOs are both exported and imported by active mechanisms described in detail elsewhere (Van Der Heide, Hoekman, & Smidt, 2004).

There are many biological functions ascribed to FOXOs, for example, they are known to participate in processes as disparate as cellular metabolism, differentiation and apoptosis (Accili & Arden, 2004). FOXO proteins exert their transcriptional activity by binding to DNA core regulatory sequences in their respective target genes, however, the specific functions of FOXOs may be determined by cell-specific co-activators of transcription (Coffer & Woodgett, 1991). In adult skeletal muscle, FOXOs are known to modulate gene expression in response to metabolic perturbations (Furuyama, Kitayama, Yamashita, & Mori, 2003; Furuyama et al., 2002; Kamei et al., 2003). Indeed, during skeletal muscle atrophy, Lecker et al. (2004) reported an increase in FOXO1 mRNA in addition to several other atrophy related genes. Together, these studies suggest that FOXOs may have specific roles in the adaptive

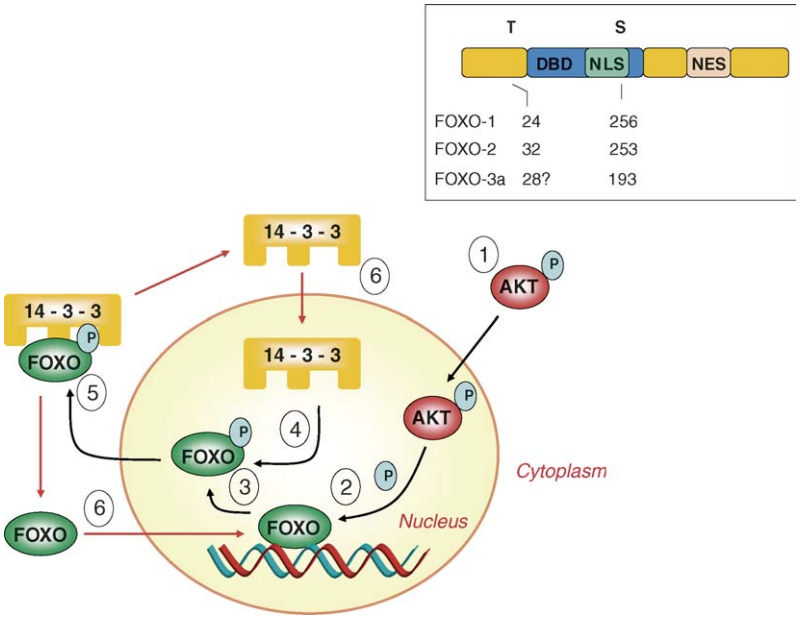

Fig. 2. Mechanism of FOXOs regulation by AKT. (1) Activation of AKT1 leads to the (2) inactivation of FOXOs causing their (3) release from DNA and (4) binding to 14-3-3. (4) FOXOs-14-3-3 complexes are (5) exported from the nucleus and once dephosphorylated, likely by inhibition of AKT 1, (6) are imported back to the nucleus. Inset: Putative AKT phosphorylation sites in FOXOs. Phosphorylation of FOXOs by AKT results in their inactivation thereby preventing their nuclear import and maintenance of their cytoplasmic localization. This effect is presumably due to the phosphorylation in the Ser residue that blocks the nuclear localization signal. (DBD = DNA-binding domain, NLS = nuclear localization signal, NES = nuclear export signal).

responses of skeletal muscle to various stimuli and may play a role in the progression of muscle atrophy.

3.1. Regulation of muscle atrophy: novel roles of AKT signaling to FOXOs and regulation of Atrogin-1(MAFbx) and MuRF-1 gene expression

Two recent studies have broadened our understanding of the atrophy process in skeletal muscle. These studies began to dissect a novel function of the AKT1/FOXOs signaling network. AKT1, apart for its well-characterized role in hypertrophy, is now shown to be an effector of anabolic signals that actively prevent muscle atrophy by inhibiting the activity of FOXOs, which in turn, appear to control the expression of atrogin-1(MAFbx) and MuRF-1. Such studies relied on very elegant experimentation to systematically study the interaction between AKT1 signaling and FOXOs in the progression of atrophy in vivo and in vitro.

In the first report, Sandri et al. (2004) have established a model of in vitro atrophy in which differentiated myotubes were either deprived of nutrients or induced to atrophy by treatment with the synthetic glucocorticoid dexamethasone + T3 (referred as dexamethasone). Following 6 h of nutrient deprivation, a 60% reduction in myotube diameter was observed together with a 2.5-fold increase in atrogin-1(MAFbx) mRNA. Similarly, dexamethasone treatment resulted in a 40% reduction in myotube size together with a ∼3-fold increase in atrogin-1(MAFbx) mRNA. These two conditions are in agreement with the in vivo response to either treatment (Jagoe, Lecker, Gomes, & Goldberg, 2002; Lecker et al., 2004), which suggests that the muscle culture system is a valid model to study skeletal muscle atrophy. Not surprisingly, during these two catabolic states in which myotube atrophy occurs, an inhibition of AKT1 phosphorylation was observed indicating that during myotube atrophy, there is a decrease in the activity of signaling molecules involved in protein synthesis.

Consistent with the inhibition of AKT1 phosphorylation, and because FOXOs are downstream targets of AKT1, phosphorylation of FOXOs decreased accordingly during myotube atrophy. Such decrease in phosphorylation was accompanied by an increased content of FOXO1 and FOXO3a proteins in the nuclear compartment, suggesting that upon AKT inhibition, dephosphorylation of FOXOs caused their re-localization to the cell nucleus where they can modulate the expression of target genes. Conversely, treatment with the anabolic agent IGF-1, which is known to increase AKT1 phosphorylation, resulted in increased phosphorylation of FOXO1 and FOXO3a and suppressed atrogin-1(MAFbx) mRNA expression providing further support to the role of AKT1 in the regulation of FOXOs.

The actions of IGF-1 are likely mediated by several effectors, thus in order to determine whether AKT1 activity was sufficient to mediate the effects of IGF-1 on the phosphorylation and inhibition of FOXOs and atrogin-1(MAFbx) expression, a constitutively active (c.a.) AKT construct was transfected into the myotubes treated with dexamethasone. Similar to IGF-1 treatment, c.a.AKT prevented the dexamethasone-induced dephosphorylation of FOXOs and consequently blocked the induction of atrogin-1(MAFbx), demonstrating that the activity of AKT 1 is sufficient to inactivate FOXOs and reduce atrogin-1(MAFbx) expression. In addition, these results provided evidence suggesting that IGF-1 signaling via AKT1 could prevent the induction of atrogin-1(MAFbx) by dexamethasone via inhibition of FOXOs.

To further explore if the inhibition of FOXOs was directly involved in the regulation of atrogin-1(MAFbx) expression, a c.a.FOXO3a that could not be inactivated by AKT1 was transfected into the myotubes. Expression of wild-type (w.t.) FOXO3a and c.a.FOXO3a caused a six-fold increase in atrogin-1 mRNA accumulation. IGF-1 treatment, similar to its effects in reversing atrophy, could reverse the increase in atrogin-1 (MAFbx) in the w.t.FOXO3a but not in the c.a.FOXO3a-transfected myotubes. Moreover, expression of c.a.FOXO3a for 48 h induced a 50% reduction in myotube diameter and, a dominant negative (d.n.) version of FOXO3a that cannot bind DNA, prevented atrogin-1 (MAFbx) expression and blocked dexamethasone-induced myotube atrophy. These results indicate that the IGF-1-induced AKT1 signaling effects on atrogin-1 (MAFbx) expression are mediated at least by FOXO3a, and that FOXO3a plays a key role in the progression of atrophy as it is sufficient to induced reductions in myotube size, block IGF-1 inhibition of atrogin-1(MAFbx) expression and dexamethasone-induced atrophy.

Further experiments aimed at testing the role of AKT1 activity on atrogin-1(MAFbx) expression in vivo were carried out using c.a.AKT1 together with an atrogin-1(MAFbx) promoter-reporter construct termed *3.5AT1*. In adult mouse skeletal muscle, atrogin-1(MAFbx) gene expression during food deprivation was shown to be due to an increased transcription as determined by an increase in luciferase activity of the *3.5AT1* construct. In this condition, co-transfection of c.a.AKT1 and *3.5AT1* reporter showed that c.a.AKT1 completely blocked the increase in atrogin-1(MAFbx) promoter activity previously shown to occur during food deprivation, which demonstrates that AKT1 activity is sufficient to downregulate atrogin-1(MAFbx) expression during food deprivation-induced atrophy of adult skeletal muscle.

Finally, to further validate the in vitro results in vivo of FOXO3a as mediator of the effects of AKT1 activity on atrogin-1(MAFbx) expression, co-transfection of either w.t.FOXO3a or c.a.FOXO3a together with the *3.5AT1* reporter was carried out in adult mouse skeletal muscle. Co-transfection results showed that during food deprivation, w.t.FOXO3a and c.a.FOXO3a resulted in a 3- and 20-fold induction in reporter activity, respectively. Moreover, targeting FOXO1 or FOXO3a with an RNA interference construct that blocks the function of these FOXOs resulted in a complete inhibition of *3.5AT1* reporter activity in 24-h food-deprived muscles together with a 50% decrease in protein levels of FOXO1 and FOXO3a. Indeed, expression of c.a.FOXO3a resulted in significant muscle atrophy at 8 and 14 days post-transfection with individual fibers displaying 35 and 58% smaller cross-sectional areas than control fibers, respectively. These findings demonstrate that FOXO3a mediates the effects of AKT on atrogin-1(MAFbx) expression and that alone, FOXO3a can induce a dramatic decrease in muscle fiber size. Altogether, these results provide direct evidence indicating that activation of AKT1 by IGF-1 leads to inactivation of FOXO3a and repression of atrogin-1(MAFbx) gene expression and that AKT1 alone is sufficient to modulate atrogin-1(MAFbx) gene expression.

In another study, Stitt et al. (2004) have undertaken a similar approach to expand the knowledge about AKT1 signaling on the activities of FOXOs and the regulation of both E3 ligases atrogin-1(MAFbx) and MuRF-1. Similarly, they have found that AKT1 exerts control over both atrogin-1(MAFbx) and MuRF-1 expression by modulating the activity of FOXO1. In this study, dexamethasone treatment of culture myotubes for 24 h resulted in ∼50% reduction in myotube diameter and a net loss of contractile protein content. An upregulation of both atrogin-1(MAFbx) and MuRF1mRNA was detected concurrent with dexamethasone-induced myotube atrophy. Interestingly, the upregulation of atrogin-1(MAFbx) expression was more dramatic than that of MuRF-1.

In agreement with the previous report, addition of IGF-1 to cultured myotubes for 24 h could prevent dexamethasone-induced atrophy and the upregulation of both atrogin-1(MAFbx) and MuRF1. These results demonstrate that IGF-1 could exert a powerful anti-atrophy effect, presumably by activating a signaling network comprising PI-3K and AKT1. Indeed, the effects of IGF-1 were mediated by PI-3K and AKT1 signaling, as myotubes stably transfected with vectors containing either c.a.AKT1 or c.a.PI-3K constructs and treated with dexamethasone were refractory to dexamethasone-induced increase in both atrogin-1(MAFbx) and MuRF-1 mRNA.

To further understand the mechanism by which activation of AKT1 inhibited the expression of atrogin-1(MAFbx) and MuRF-1, a series of experiments were carried out to test: (a) if the phosphorylation of FOXO3a and FOXO1 was modulated after treatments with IGF-1 and/or dexamethasone, (b) whether the ability of AKT1 phosphorylation to inhibit FOXO1 was responsible for its cytoplasmic localization and (c) whether FOXO1 could control the expression of atrogin-1(MAFbx) and MuRF-1.

Phosphorylation of FOXO3a and FOXO1 was detected upon IGF-1 treatment, however, dexamethasone failed to induce the dephosphorylation of the transcription factors in the IGF-1 treated myotubes. In order to test whether IGF-1-induced AKT1 activity mediated the lack of effect of dexamethasone on FOXO1 translocation to the nucleus, myotubes were transfected with either a w.t.FOXO1 or a c.a.FOXO1 construct. As expected, w.t.FOXO1 was predominantly cytoplasmic but the c.a.FOXO1 was mainly nuclear. Consistent with the previous knowledge that AKT1 modulates FOXOs localization and that AKT1 is regulated by PI-3K, specific inhibition of PI-3K resulted in the nuclear translocation of w.t.FOXO1 but had no effect on c.a.FOXO-1 demonstrating that nuclear translocation of at least FOXO1 is PI-3K dependent and could be mediated by AKT1 activity. Then to determine whether FOXO1 could modulate the expression of atrogin-1(MAFbx) and MuRF-1, w.t.FOXO1 and c.a.FOXO1 were transfected in myotubes and treated with dexamethasone, IGF-1 or a combination of both. Surprisingly, neither w.t.FOXO1 nor c.a.FOXO1 affected the baseline expression of atrogin-1(MAFbx) or MuRF-1. Similarly, dexamethasone had no effect on the expression of atrogin-1(MAFbx) or MuRF-1 in the presence of either construct. IGF-1, however failed to block the dexamethasone-induced changes in gene expression of both atrophy genes in the presence of c.a.FOXO1 but it could still block gene expression in the presence of w.t.FOXO1. These results indicate that while FOXO1 is not sufficient to induce transcription of atrogin-1(MAFbx) and MuRF-1, inactivation of FOXO1 is required for IGF-1 to block dexamethasone-induced expression of atrogin-1(MAFbx) and MuRF-1.

Finally, to validate the previous findings in an in vivo model of atrophy, denervated muscles were injected with IGF-1 and its effects on muscle mass, together with atrogin-1(MAFbx) and MuRF-1 expression, were determined. IGF-1 injection of denervated muscles partially ameliorated atrophy (10% loss versus 30% loss in the saline-treated muscles). Importantly, the muscle sparing effect of IGF -1 was nicely correlated with a marked reduction in the expression of both atrogin-1(MAFbx) and MuRF-1 as compared with the saline treated group. These findings indicate that administration of IGF-1 could ameliorate denervation-induced atrophy concomitant with the marked inhibition of atrogin-1 (MAFbx) and MuRF-1.

4. Summary and perspectives for future research

The specific goal of the present review was to discuss recent findings describing the mechanisms involved in the regulation of skeletal muscle atrophy. The elegant investigations of Sandri et al. (2004) and Stitt et al. (2004) and their co-workers have improved

our understanding of the mechanisms involved this process. Their data identifies AKT1 as a key regulator of atrogin-1(MAFbx) and MuRF-1 expression via FOXOs during the progression of skeletal muscle atrophy. This is a new function of AKT1 in skeletal muscle that complements its previously known role as a mediator of muscle hypertrophy, and highlights its role as a nodal point for the integration of both anabolic and catabolic signals that lead either to the increase or decrease in skeletal muscle mass. This central role of AKT1 represents an important mechanism which ensures that the cell manages its energy utilization in an efficient manner as it would be wasteful for a cell to spend energy synthesizing proteins at the same time that proteins are wasted away by degradative processes. Therefore, the notion of AKT-1 as a "coordinator" of both processes of protein synthesis and protein degradation seems biologically sound.

However, despite these exciting new findings, a few simple but important questions have emerged. For example, during certain catabolic conditions such food deprivation and denervation, a decrease in AKT1 activity seems to take place concomitant with the occurrence of muscle atrophy. Given the importance of AKT1 signaling in the maintenance of skeletal muscle, it will be important to identify which signals are suppressing AKT1 activity during the occurrence of muscle atrophy. We have also learned that FOXOs are important factors mediating the expression of atrophy associated genes such as atrogin-1(MAFbx) and MuRF-1. However, some differences may exist between the different FOXO isoforms. For example, while FOXO3a could potently induce the expression of atrogin-1(MAFbx), FOXO1 failed to induce the expression of neither atrophy related gene in vitro (Stitt et al., 2004). This is intriguing and suggests the possibility that the different FOXO isoforms may have specific and/or dual functions as transcriptional activators and/or transcriptional repressors on various gene targets (Van Der Heide et al., 2004). In addition, the fact that FOXO1 failed to induce the expression of atrogin-1(MAFbx) and MuRF-1 is surprising as in vivo overexpression of FOXO1, which results in significant muscle atrophy, induces the upregulation of at least atrogin-1(MAFbx) and other atrophy related genes (Kamei et al., 2004). In this respect, future investigations should involve the study of FOXOs independently in the absence of the other isoforms.

Another important aspect regarding the functions of FOXOs is that the amount of atrophy induced by c.a.FOXO3a was more pronounced than that produced by atrogin-1(MAFbx) expression alone (Sandri et al., 2004). It is not clear why this happened, but it seems logical to speculate that FOXO3a may regulate a larger subset of genes involved in atrophy in addition to the regulation of atrogin-1(MAFbx) and MuRF-1 as deletion of either gene could only partially ameliorate the progression of denervation-induced atrophy in vivo by 56 and 36%, respectively (Bodine, Latres et al., 2001). Thus, it is plausible that FOXO-induced skeletal muscle atrophy is mediated by an "atrophy transcriptional program" involving several other genes in addition to atrogin-1(MAFbx) and MuRF-1 ("atrogenes") (Lecker et al., 2004). In addition, a recent report by Lee et al. (2004) demonstrated that inhibition of PI-3K lead not only to the expression of atrogin-1(MAFbx) but it also resulted in an increase in caspase-3, a protease believed to disassemble the contractile apparatus by cleaving actomyosin (Du et al., 2004) suggesting that AKT1 signaling to FOXOs may represent only a partial aspect of the signaling involved in the atrophy process. Altogether, these studies suggest that skeletal muscle atrophy may involve several steps including the cooperative degradation of muscle proteins by the various degradative systems.

Much has been learned about the mechanisms involved in the regulation of skeletal muscle mass. This information is valuable for the development of pharmacological and therapeutic therapies aimed at ameliorating the devastating effects that muscle atrophy could have in health and disease. However, the challenge that remains for both scientists and clinicians is the translation of this important molecular information from the "bench to the bedside".

Acknowledgments

Due to the specific focus of the present review, many important studies were not included in the discussion. Apologies are given to the authors of those studies for the omission their work. I would like to thank only colleagues at the Research Center for Genetic Medicine for many stimulating discussions, especially to Dr. Eric P. Hoffman for his continuous support and valuable advice.

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