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Mechanisms Stimulating Muscle Wasting in Chronic Kidney Disease: The Roles of the Ubiquitin-Proteasome System and Myostatin

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Abstract

Catabolic conditions including chronic kidney disease (CKD), cancer, and diabetes cause muscle atrophy. The loss of muscle mass worsens the burden of disease because it is associated with increased morbidity and mortality. To avoid these problems or to develop treatment strategies, the mechanisms leading to muscle wasting must be identified. Specific mechanisms uncovered in CKD generally occur in other catabolic conditions. These include stimulation of protein degradation in muscle arising from activation of caspase-3 and the ubiquitin-proteasome system (UPS). These proteases act in a coordinated fashion with caspase-3 initially cleaving the complex structure of proteins in muscle yielding fragments that are substrates which are degraded by the UPS. Fortunately, the UPS exhibits remarkable specificity for proteins to be degraded because it is the major intracellular proteolytic system. Without a high level of specificity cellular functions would be disrupted. The specificity is accomplished by complex reactions that depend on recognition of a protein substrate by specific E3 ubiquitin ligases. In muscle, the specific ligases are Atrogin-1 and MuRF1 and their expression has characteristics of a biomarker of accelerated muscle proteolysis. Specific complications of CKD (metabolic acidosis, insulin resistance, inflammation, and angiotensin II) activate caspase-3 and the UPS through mechanisms that include glucocorticoids and impaired insulin or IGF-1 signaling. Mediators activate myostatin which functions as a negative growth factor in muscle. In models of cancer or CKD, strategies that block myostatin prevent muscle wasting suggesting that therapies which block myostatin could prevent muscle wasting in catabolic conditions.

Keywords

ubiquitin-proteasome system (UPS); muscle wasting; protein degradation; chronic kidney disease (CKD); caspase-3

Introduction

Several mechanisms have been advanced to explain the muscle atrophy that occurs commonly in CKD but none have been universally accepted. The mechanisms that have been proposed are mainly based on the presence of stimuli that could activate loss of protein stores, namely a decrease in protein synthesis or an increase in protein degradation leading to loss of muscle proteins [1]. Alternatively, some nephrologists believe that the metabolic problems causing protein losses are principally due to an inadequate diet. For example, the

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diagnosis of muscle wasting in CKD patients has principally relied on the presence of hypoalbuminemia and since hypoalbuminemia occurs in children with inadequate protein intake or “kwashiorkor”, it is widely believed that hypoalbuminemia in CKD patients is synonymous with the presence of “protein malnutrition”. Since malnutrition is defined as a problem caused by an inadequate diet, it follows that the loss of protein stores and reversal of hypoalbuminemia should be corrected by simply changing the diet [1;2]. Support for the conclusion that the problem is an inadequate diet is found in reports that some patients with progressive CKD spontaneously restrict their protein intake [3]. Although a proper diet can ameliorate the loss of protein stores in patients with CKD, the loss of protein stores in CKD patients is rarely caused by malnutrition [1;4]. More importantly, assigning a critical role for an inadequate diet as the cause of lost protein stores is overly simplified because the hypothesis that protein malnutrition in CKD patient is a major cause of lost protein stores has been examined and found to be wanting: Ikizler and colleagues measured rates of protein synthesis and degradation in fasting hemodialysis patients using standard techniques of measuring the turnover of labeled amino acids [5]. Specifically, the components of protein metabolism (protein synthesis and degradation) were measured before, during and at 2 hours after completing the dialysis treatment. At each stage, protein degradation exceeded protein synthesis leading to a significant loss of body protein stores. The investigators then used the same protocol to test whether administering intravenous parenteral nutrition (IDPN) during hemodialysis would correct the abnormalities in protein turnover and hence, prevent the loss of protein stores [6]. However, the overall pattern of abnormal protein synthesis and degradation was largely unchanged except that protein metabolism improved during IDPN. The catabolic response to dialysis persisted and was still present after stopping the dialysis. In a third set of experiments, Pupim et al. repeated the protocol but in this case, they compared the results with IDPN to those that occurred when the patients were given a nutritional supplement to eat during the dialysis [7]. In this experiment as well, the oral supplement led to an improvement in protein balance but it was still negative at two hours after discontinuing the dialysis. These careful measurements not only demonstrate that hemodialysis activates catabolic responses that reduce protein stores, they also demonstrate that simply increasing protein in the diet will not eliminate the activation of catabolic pathways that cause protein losses in CKD patients. The results also provide an explanation why results from a two year long, randomized, controlled trial of responses to IDPN vs. no dietary supplementation in hemodialysis patients resulted in a negative outcome [8]. Unfortunately, the IDPN did not improve mortality, body mass index, laboratory markers of nutritional status or the rate of hospitalization associated with the IDPN supplement. In summary, in patients with CKD, hemodialysis activates protein degradation resulting in a loss of body protein stores. The loss of protein stores is not eliminated by simply altering the diet. This does not mean that the diet should be ignored; we believe that the diets of CKD patients should be regularly reviewed to ensure patients are eating sufficient amounts of protein and calories to meet their requirements [9]. In addition, when patients develop hypoalbuminemia or loss of weight there should be an investigation to uncover conditions and when found, efforts should be undertaken to correct the abnormality rather than simply recommending an increase in dietary protein [1;2].

Mechanisms Stimulating Protein Catabolism

A major effort in clinical studies of CKD patients has been to identify factors that are associated with hypoalbuminemia and other complications of kidney disease. For example, it is well documented that circulating levels of cytokines are frequently increased in patients with CKD suggesting that inflammation is the mechanism that causes hypoalbuminemia. To examine the association between circulating cytokines and hypoalbuminemia in depth, Kaysen et al. studied patients who were receiving long term dialysis in a regimented fashion of standardized treatments [10]. Each month, serum albumin as well as the circulating levels

of α -1 acid glycoprotein and ceruloplasmin were measured (representative markers of the presence of inflammation). Analysis of the results revealed that an increase in the levels of inflammatory markers measured in one month would predict that the serum albumin would be lower in the succeeding month. It was also determined that variations in the diet exerted only a minimal influence on changes in serum albumin. The authors concluded that the presence of inflammation is the most important factor associated with the development of hypoalbuminemia and that changes in the diet had only a limited influence on serum albumin in hemodialysis patients. The experiment did not uncover how inflammatory cytokines cause a decrease in serum albumin and the results suggest that hypoalbuminemia acts as a “biomarker” of inflammation.

There are other causes of hypoalbuminemia besides inflammation. For example, CKD is often complicated by the development of metabolic acidosis which can lead to hypoalbuminemia. In this case, the diet plays an important role because the amount of dietary protein contributes to the generation and maintenance of metabolic acidosis. Specifically, the metabolism of sulfa-containing amino acids or phosphorylated proteins and lipids yields acids which accumulate when kidney function decreases [11]. Therefore, a protein-rich diet will generate metabolic acidosis in patients with CKD, negatively affecting serum albumin. Proof that metabolic acidosis exerts a negative influence on albumin was obtained when normal adults were given ammonium chloride (NH_4Cl) to induce acidosis [12]. This led to a significant decrease in the ability of the subjects to synthesize albumin. In another experiment, Movilli and colleagues studied hemodialysis patients with metabolic acidosis before and after they were treated with supplements of sodium bicarbonate (NaHCO_3). After 3 months, correction of the metabolic acidosis was associated with significant improvements in the serum albumin concentration [13]. The NaHCO_3 treatment also decreased protein catabolism but only in patients who did not have high levels of circulating cytokines (i.e., inflammation). Notably, the benefits of the NaHCO_3 supplements were found to be independent of dietary protein or the intensity of dialysis.

Catabolic pathways that simulate loss of protein stores

All intracellular and extracellular proteins are continually “turning over”, being degraded to amino acids and replaced by the synthesis of new proteins [14]. Specific proteins in the nucleus, cytoplasm, endoplasmic reticulum or mitochondria are degraded at vastly different rates varying from minutes (e.g., some regulatory enzymes or transcription factors) to days or weeks for skeletal muscle proteins such as actin and myosin or months for certain proteins (e.g. hemoglobin). This variability in half lives of different proteins is possible because proteins are degraded at different rates by proteolytic processes that are highly regulated. Without such regulation, there would be chaotic changes in protein half-lives, causing serious impairment in cellular and organ functions. The importance of regulated protein degradation is emphasized because the amount of protein that is metabolized each day is very large, amounting to 3.7 to 4.7 g/kg/day. Since only 20% of muscle weight is protein, the amount of protein that is synthesized and degraded each day in a 70 kg adult would be at least 1 kg of muscle! For these reasons, a small but sustained decrease in protein synthesis or increase in protein degradation would cause a marked loss of protein stores [14]. Considering the importance of regulating the degradation of so many proteins, it is surprising that only one highly regulated proteolytic system, the ATP-dependent, ubiquitin-proteasome system (UPS), is responsible for the degradation of the majority of intracellular proteins in all tissues [14].

The ubiquitin-proteasome system

The influence of CKD on muscle protein metabolism has concentrated on changes in protein degradation because a decrease in protein synthesis induced by CKD is much less prominent than an increase in protein degradation [14–16]. Specific steps in the degradation of proteins by the UPS have been identified [14;17]. Before degrading a protein, the UPS first “tags” it by conjugating the protein to ubiquitin (Ub), a small protein that is a member of the heat-shock protein family. This Ub conjugation system dooms the substrate because the process is repeated until a chain of Ub’s are attached to a substrate protein (Figure 1). The chain of Ub’s is recognized by the 26S proteasome leading an unfolding of the protein and ultimately, its degradation in the central core of the 26S proteasome [14;17]. As emphasized, the process of conjugating the protein substrate to Ub is highly regulated: it begins with activation of Ub by an ATP-requiring step that is accomplished by a single E1 isoform (Ub-activating enzyme). The activated Ub can then interact with one of the 20–40 isoforms of E2 Ub-carrier proteins. These initial steps do provide some specificity to the degradation of a specific protein by the UPS because only a specific class of proteins can interact with the E2 Ub-carrier protein. In addition, each E2 Ub-carrier protein interacts only with a specified group of E3 ubiquitin ligases and hence, a specific E3 Ub ligase is responsible for ligating Ub to the substrate protein. The key to achieving specificity lies in the E3 Ub ligases: there are more than 1000 of the E3 enzymes and each recognizes only a specific protein substrate (or possibly a class of proteins). Following recognition of the protein to be degraded, the activated Ub is transferred to the protein. Initially, the activated Ub is transferred to a lysine in the substrate protein but subsequently, other Ub’s are transferred to lysines that are present in the Ub that was attached to the substrate protein. The transfers continue until 5 Ub’s are present as a chain which acts to identify a substrate protein that will be degraded. The degradation occurs in the 26S proteasome, a very large organelle consisting of >60 proteins organized into two particles, a 20S, central, barrel-shaped particle plus 19S regulatory particles at either or both ends of the central, 20S particle [17]. The 19S particles on each end are capable of recognizing the polyubiquitin chain and in the presence of sufficient ATP, the proteasome cleaves the Ub chain from the substrate protein and recycles the Ub’s. In addition, the 26S proteasome unfolds the substrate protein and translocates it into the 20S particle where it is degraded to peptides. The peptides released from the proteasome are rapidly converted to amino acids by cytosolic peptidases. This completes the degradation of substrate proteins. The importance of these reactions is underscored by the 2004 Nobel Prize in Chemistry awarded to Avram Hershko, Aaron Ciechanover and Irwin Rose for their discovery of Ub and its biochemistry.

Details of the activity of the UPS are provided because it is the major proteolytic system that is activated by catabolic conditions to degrade muscle proteins. Specifically, different catabolic conditions accelerate the conjugation of Ub to substrate proteins in muscle, stimulating degradation of the proteins. The rise in Ub conjugation and protein degradation proceeds for two reasons: first, there is increased expression of the mRNAs and proteins of critical components of the degradation process, including both Ub and subunits of the 26S proteasome. Second, the acceleration of muscle protein degradation in muscle involves an increase in the expression and activity of two muscle-specific, E3 Ub ligases, Atrogin-1 (also known as MAFbx) and MuRF-1. Evidence for their importance includes a 8-to 20-fold higher expression in muscles of rodent models of muscle wasting conditions; the increase in their expression is correlated with higher rates of protein degradation [17–20]. Mechanisms that regulate the activation of these E3 Ub-conjugating enzymes has been intensively studied and at least two regulatory factors have been identified, the forkhead transcription factors (FoxO) and the inflammatory transcription factor, NFκB. FoxO and NFκB activate the promoters for Atrogin-1/MAFbx and MuRF-1 respectively and an increase in the expression of these specific E3 Ub ligases stimulates muscle protein degradation in the UPS [17;18;21].

Caspase-3 accelerates protein degradation in the UPS

A loss of contractile proteins (approximately 2/3 of the protein present in muscle) is largely responsible for the disability of patients suffering from muscle wasting [22]. How does activation of caspase-3 in muscle influence protein wasting? As noted, the UPS is responsible for the degradation of the bulk of cellular proteins in all cells, it readily degrades actin, myosin, troponin or tropomyosin but exhibits only limited proteolytic activity towards these same proteins when they are present in the complex structure of muscle proteins [23]. Therefore, another protease must initially cleave the proteins in muscle in order to produce substrates for degradation by the UPS. We have found that activated caspase-3 accelerates muscle protein degradation in two ways. First, it performs an initial cleavage of actomyosin and myofibrils producing protein fragments that are rapidly degraded by the UPS [19]. This proteolytic response can be detected because the action of caspase-3 on muscle proteins leaves a “footprint”, a 14 kD C-terminal fragment of actin. The 14 kD actin fragment is most easily detected in the insoluble fraction of a muscle biopsy because the fragment is rapidly degraded by the UPS when it is present in the soluble fraction of muscle. We speculate that the reason for the difference between recovery from the soluble and insoluble fractions is that the 14 kD actin fragment in the insoluble fraction is not degraded because the activity of the UPS is “hindered”, reducing contact with the cellular actin.

In addition to caspase-3 activity in muscles of experimental models of catabolic conditions, there is evidence that caspase-3 cleaves muscle proteins in patients with CKD. First, an increase in the levels of the 14 kD actin fragment was found in muscle biopsies of patients with CKD or those who had a burn injury to other parts of the body [24]. The 14 kD actin fragment was also found in muscles of patients who had a measurable loss of muscle mass in a leg and had undergone surgery to replace a hip. In these patients, the level of the 14 kD actin fragment in muscle biopsies was highly correlated ($r = 0.78$) with the measured rate of protein degradation in the same muscle. Second, the level of the 14 kD actin fragment can respond to therapy: it was significantly reduced in muscle biopsies of CKD patients who had completed 18 weeks of an exercise program consisting of endurance exercises. If these results were expanded, muscle levels of the kD actin fragment might serve as a biomarker of accelerated muscle protein degradation.

The second mechanism by which caspase-3 increases muscle proteolysis is that it stimulates proteolytic activity of the proteasome. In this case, the activated caspase-3 cleaves specific subunits of the 19S particle of the proteasome to increase proteolytic activity [25]. Presumably, cleavage of the subunits opens the entrance into the 20S proteasome, permitting more of the substrate proteins to enter the 20S fragment where they are degraded (see above). In summary, in rodent models and in patients responding to catabolic conditions, caspase-3 exerts two functions that increase muscle protein degradation: 1) it performs an initial cleavage of muscle proteins, producing more substrates for degradation by the UPS; and 2) it stimulates proteolytic activity of the proteasome. Together, these properties exert a “feed-forward” stimulation of muscle proteins when caspase-3 is activated.

Increased activity of calpains has been suggested to be another mechanism that increases myofibrillar protein catabolism. Calpains are calcium-dependent, cysteine proteases that are active in muscular dystrophy or sepsis-induced muscle wasting [26]. Their role in breaking down muscle proteins in other conditions is unclear since the muscle wasting that occurs in response to uremia or impaired insulin/IGF-1 signaling is unaffected when calpain activity is inhibited [16;19].

Muscle proteolysis can be detected

As noted, the action of caspase-3 in degrading muscle proteins can be detected from finding the 14 kD actin fragment in a muscle biopsy. There are other methods of detecting activation of the UPS. In muscles of patients with trauma, cancer, CKD or sepsis, the level of mRNAs encoding Ub and subunits of the proteasome are increased [27–30]. For example, CKD patients being treated by chronic ambulatory peritoneal dialysis (CAPD) were studied during a year-long, randomized trial designed to determine the influence of correcting metabolic acidosis [31]. Elimination of the metabolic acidosis was accompanied by an increase in body weight and muscle mass plus a reduction in hospitalization days. Using a similar protocol, we found that correction of even mild metabolic acidosis over a period of about one month was associated with a decrease in the mRNA levels of Ub [27]. Since animal models of accelerated muscle proteolysis exhibit increased levels of Ub mRNA in muscle, the demonstration that correcting metabolic acidosis lowered Ub mRNA is compatible with decreased activity of the UPS. Thus, there is evidence that patients with CKD humans activate the same proteolytic pathways uncovered in rodent models of accelerated protein degradation [24].

Factors triggering muscle wasting in CKD and other catabolic states

It is well known that glucocorticoids (GC) can cause muscle wasting but the mechanism for acceleration of protein degradation has not been fully identified. In one model, supraphysiologic doses of GC activate the UPS and increase the levels of mRNA encoding Ub and subunits of the proteasome. The mechanism causing this response is not known because there are no glucocorticoid receptor elements in the promoters of these genes so the reason for the increase in mRNA levels is unknown. For a physiologic level of GC, the basis for enhancement of protein degradation has been identified; it is a non-genomic mechanism that activates the UPS [32]. It was found that an increase in circulating GC leads to an interaction between the glucocorticoid receptor and the p85 subunit of phosphatidylinositol 3-kinase (PI3K). The interaction results in reduced ability to phosphorylate Akt, indicating there is impairment in insulin/IGF-1 intracellular signaling (i.e., insulin resistance). These results therefore explain why the presence of metabolic acidosis, an excess angiotensin II or the development of diabetes or insulin resistance will not cause muscle protein degradation unless GC are present [33–35]. In summary, conditions associated with impaired responses to insulin or IGF-1 result in suppression of the phosphatidylinositol 3-kinase/Akt (PI3K/Akt) signaling pathway. Decreased activity of this pathway stimulates muscle wasting by the following mechanism: a low value of p-Akt decreases the phosphorylation of downstream kinases, mTOR and s6 kinase and hence, suppresses protein synthesis. A decrease in p-Akt also reduces phosphorylation of the family of forkhead transcription factors (FoxO1, 3, and 4). When these factors are not phosphorylated, they can translocate to the nucleus to stimulate transcription of the muscle-specific E3 Ub ligases, Atrogin-1 and MuRF-1. The expression of these E3 Ub ligases stimulates muscle protein degradation in the UPS [18].

Recently, we identified another factor that contributes to muscle wasting in CKD, namely abnormal function of satellite cells. These cells function as muscle “stem” cells and under normal conditions, they are quiescent, remaining under the basal lamina of myofibrils. In response to muscle injury or muscle protein wasting, satellite cells proliferate and differentiate into myofibrils that act to repair lost muscle mass [36;37].

Therapeutic strategies for preventing muscle wasting

As noted in preceding paragraphs, much has been learned about mechanisms that regulate protein degradation in muscle and how catabolic conditions accelerate the degradation of muscle proteins. Recently, we found a potential strategy for interrupting the protein

catabolic responses. The strategy is to inhibit myostatin, a member of the transforming growth factor- β (TGF- β family of secreted proteins). Unlike TGF- β , myostatin is predominantly expressed in skeletal muscle (low levels of myostatin are detectable in cardiac muscle). In skeletal muscle, there is a precursor of myostatin, premyostatin, which is cleaved to produce promyostatin and promyostatin consists of a propeptide plus myostatin. Myostatin is released from the propeptide when there is a loss of muscle protein or an excess in free radicals or a decrease in pH. The released myostatin binds to a high-affinity, type-2 activin receptor (ActRIIB) that is present on muscle membranes. Activation of this receptor initiates phosphorylation of SMAD transcription factors (Figure 2). Notably, other members of the TGF- β family (e.g., activin A) can also bind to the ActRIIB receptor and stimulate the same intracellular signaling pathway.

Genetic evidence has demonstrated that myostatin plays a pivotal role in regulating skeletal muscle mass and function. For example, deletion of the myostatin gene in mice produces an approximate 30% increase in both the size and number of skeletal muscle fibers [38;39]. Alternatively, a “loss-of-function” mutation in the myostatin gene in mice, cattle, sheep, dogs and at least one human causes an enormous increase in muscle mass [36;39;40]. There also is evidence that myostatin mutations influence athletic performance: whippet dogs with a single copy of the myostatin mutation are among the fastest dogs in competitive racing [41]. But, whippets bearing two copies of the same mutation develop such large muscles they are unable to win. Results from elite thoroughbred horses also suggest that myostatin polymorphism provides a decided advantage for racing against other horses [42].

Myostatin and its signaling pathway are increased in conditions characterized by muscle wasting: in aging adults or bed-ridden patients there are high levels of myostatin in muscle as there is in patients with AIDS, renal failure or heart failure [43–46]. These responses suggest that blocking myostatin or the activin A pathway could improve muscle size and function in catabolic conditions. Indeed, blocking myostatin responses has been accomplished in mouse models of cancer. Devising such a treatment is of special interest because as many as 80% of patients with advanced cancers develop muscle wasting and 25% of cancer-related deaths can be ascribed to cachexia. A major reason for the loss of muscle mass is that protein degradation is stimulated by impaired p-Akt signaling by mechanisms that activate both caspase-3 and the UPS to stimulate muscle proteolysis [39]. In experimental models of cancer, myostatin responses were blocked using an ActRIIB decoy receptor as the strategy to block myostatin activity [39]. Specifically, Zhou et al. administered an ActRIIB decoy receptor to mice that had been transplanted with colon-26 carcinoma or with human TOV-21G ovarian carcinoma, human G361 melanoma, or inhibin-deficient mice that spontaneously develop gonadal tumors. In each case, muscle wasting was prevented. An especially important finding was that the treatment produced a significant increase in survival of the cancer-bearing mice even though the treatment did not reduce the size of the tumors. Secondly, treatment with the ActRIIB reagent blocked muscle protein degradation as evidenced by increased muscle levels of p-Akt and p-FoxO3a; there also was a decrease in the transcription of Atrogin-1/MAFbx and MuRF1 (see above). Thirdly, the functions of satellite cells improved as assessed by an increase in their proliferation. Finally, the treatment blocked muscle atrophy even though circulating levels of TNF- α , IL-1 β and IL-6 in the colon-26 cancer model were not suppressed [39]. The latter observation suggests that the ActRIIB decoy receptor was acting independently of mechanisms that depend on activation by inflammatory cytokines.

In addition to blocking the muscle wasting that occurs in models of cancer, we found that blocking myostatin is also effective in preventing the muscle protein losses that are induced by CKD. Since the muscle level of myostatin is increased by CKD, we studied its contribution to muscle wasting [36;46]. In these experiments, uremia in mice was induced

by subtotal nephrectomy and feeding a 40% protein diet. These mice had BUN values >80 mg/dL and characteristics of uremia [15;20;46]. Mice with CKD were paired for BUN and weight and were pair-fed for 4 weeks. One CKD mouse received subcutaneous injections of an anti-myostatin peptibody consisting of a genetically engineered myostatin-neutralizing peptide fused to Fc. The paired CKD mouse was injected with the diluent while pair-feeding was continued. The CKD mice that were treated with the anti-myostatin peptibody had substantially lower levels of myostatin in muscle. They also had an increase in body weight and muscle mass that could be accounted for by reduced protein degradation and increased protein synthesis plus improvements in satellite cell function. Notably, these positive responses were independent of dietary factors since all the treated mice had CKD and were paired with the control CKD mice.

In contrast to the results in mice with cancer and treated with the ActRIIB decoy receptor, inhibition of myostatin in muscles of CKD mice led to a decrease in circulating levels of inflammatory cytokines and especially, IL-6. The mechanism underlying the decrease in circulating cytokines was investigated in cultured C2C12 myotubes (a model of events occurring in myofibrils). When we treated the myotubes with TNF- α there was an increased in myostatin production. But, when muscle cells were treated with myostatin, there was increased production of IL-6. Since IL-6 can stimulate the degradation of IRS-1 that results in insulin/IGF-1 signaling, the responses to myostatin suggest a mechanism that continues to stimulate muscle protein degradation. Consequently, blocking myostatin can suppress IL-6 and this response will improve IGF-1 intracellular signaling and block muscle protein catabolism.

Conclusion

Chronic kidney disease, cancer and certain other catabolic disorders are characterized by activation of protein degradation in muscle, a response that increases the risk of morbidity and mortality. These conditions are also associated with inflammation, impairment in insulin/IGF-1 signaling and increased glucocorticoid production, factors that act to stimulate muscle protein degradation. The physiologic and molecular mechanisms that cause muscle protein wasting have been largely defined and there is growing evidence that myostatin plays a predominant role in stimulating muscle protein losses. Experimental results provide convincing evidence that finally strategies may be developed to intervene and prevent or reverse muscle wasting in catabolic conditions. The strategies are directed at blocking myostatin signaling in muscle and if successful, there could be effective means of preventing the loss of muscle mass. Although more information about the pathophysiology and pharmacology of muscle wasting is needed, myostatin inhibitors may prove to be a useful ally against muscle wasting in catabolic conditions.

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Fig 1. Ubiquitin-Proteasome System

The ubiquitin-proteasome system (UPS) is responsible for the identification and degradation of intracellular proteins in a markedly specific fashion. Substrate proteins to be degraded are first conjugated with a chain of 4–5 ubiquitins (Ub) that is recognized by the proteasome via an ATP dependent process. The enzymes that mediate this process are Ub-enzymes, E1 and E3 and the ubiquitin carrier protein, E2. In muscle, specific E3 ubiquitin ligases, MuRF-1 and Atrogin-1, provide distinct specificity by recognizing and marking proteins that are destined for protein breakdown. The chain of Ub that marks a protein substrate can be recognized by the 26S proteasome which uses ATP to remove the Ub chain, unfold the protein and inject it into the proteasome. The ubiquitins are recycled while the protein is rapidly degraded by the proteasomes. Finally, the peptides released during degradation are degraded by peptidases in the cell cytoplasm or used in antigen presentation.

Fig 2. Myostatin Signaling and Muscle Wasting

Myostatin is a member of the transforming growth factor β (TGF- β) family of proteins. It is released from its “latent complex” by proteolysis. The liberated myostatin can then bind to a type-2 activin receptor present on the muscle membranes. This sequence leads to recruitment and activation of a type I activin receptor transmembrane kinases, ALK 4 and ALK5, which phosphorylates Smad2 and Smad3. After recruitment of Smad4, the Smad complex binds to a Smad-binding element, SBE. The complex enters the nucleus and stimulates transcriptional changes in downstream genes that are responsible for muscle protein degradation. Concurrently, myostatin/activin binding to the ActRIIB receptor downregulates Akt activity, diminishing FoxO phosphorylation so that FoxO enters the

nucleus to activate E3 ubiquitin ligases, MuRF-1 and Atrogin-1. As shown in Figure 1, increased expression of these E3 Ub ligases stimulates muscle protein degradation.

Reference:

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