Myostatin: a therapeutic target for skeletal muscle wasting

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Purpose of review

This review discusses recent developments in myostatin research, focusing on the basic actions of myostatin on skeletal muscle, the identification of key regulatory elements of the myostatin pathway, and the promise of myostatin as a therapeutic target in muscle-related disorders.

Recent findings

In addition to a well-characterized role in muscle development, recent research advances have solidified the importance of myostatin in adult muscle, although questions remain. A number of possible regulatory proteins for myostatin have been identified, showing a complex picture of myostatin regulation that requires clarification. The identification of an antimyostatin monoclonal antibody (JA16) shows the promise of myostatin as a target for muscle-wasting disorders; the antibody has already been shown to increase muscle mass in healthy older mice and muscle function in postnatal *mdx* mice.

Summary

Since its discovery in 1997, myostatin has quickly been established as a key regulator of skeletal muscle mass. Recent developments strengthen the idea that myostatin will be an important therapeutic target for muscle-wasting-related disorders, and as more details of myostatin regulation and its mechanisms of action are clarified, myostatin will continue to dominate the skeletal muscle development and muscle-wasting literature.

Keywords

GDF-8, skeletal muscle atrophy, skeletal muscle development, skeletal muscle mass, transforming growth factor beta superfamily

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Abbreviations

DMD	Duchenne muscular dystrophy
FLRG	follistatin-related gene
GDF-8	growth and differentiation factor 8
hSGT	human small glutamine-rich tetratricopeptide repeat-containing protein
TGF-β	transforming growth factor beta

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Introduction

Despite its relatively short history, myostatin has quickly become an established target of study for skeletal muscle researchers interested in identifying mechanisms of muscle development and therapies for muscle-related disorders. First reported in 1997 by McPherron *et al.* [1], myostatin (growth and differentiation factor 8; GDF-8) was identified in mice as a transforming growth factor beta (TGF-β) family member that acts as a negative regulator of skeletal muscle growth. Soon after the initial report of myostatin's discovery, several groups identified mutations in the myostatin gene in naturally bred 'double-muscled' cattle breeds [2–5], providing additional evidence for a critical role for myostatin in muscle development, and thus establishing myostatin as the key target it has become for muscle researchers. Although not reviewed here, myostatin also appears to influence adipose tissue [6–8].

Identifying how myostatin influences skeletal muscle and what processes regulate myostatin expression and activity have dominated the myostatin literature in the past few years. Following up their initial discovery, Lee and McPherron [9] established putative myostatin receptors (activin type II receptors A and B) and negative regulators (the myostatin propeptide and follistatin), and formalized a basic model of myostatin regulation. Researchers have begun exploiting this information, providing promising preliminary data about the potential of myostatin as a therapeutic target for muscle-wasting-related disorders. In the present review, we chart the recent literature on how myostatin regulates muscle growth, discuss the latest work on the regulation of myostatin expression and activity in skeletal muscle, and outline the promise of myostatin as a therapeutic target for muscle wasting. We focus on literature from 2002–2003; previous excellent reviews of myostatin are available [10•,11,12].

Mechanisms of action

Although much work remains, significant advances have been made in understanding how myostatin impacts skeletal muscle. A primary means by which myostatin negatively regulates muscle growth is by suppressing myoblast proliferation [13–15] through the inhibition of cell cycle progression [13,16•]. In embryonic myogenesis, myostatin regulates myoblast number [13]. In adult muscle, myostatin is specifically expressed in the regenerative satellite cells, and myostatin null mice have greater numbers of both satellite cells per muscle fiber and activated satellite cells [16•].

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How myostatin works to negatively regulate satellite cell activity is starting to become known. Myostatin, in an autocrine fashion, appears to inhibit the progression of satellite cells from the G_0 or G_1 cell cycle phase to the S phase by upregulating cyclin-dependent kinase inhibitors (e.g. p21, p27 and p53) thereby downregulating cyclin-dependent kinases (e.g. Cdk2), and thus inhibiting the withdrawal from the cell cycle that is necessary for differentiation [13,16•,17,18••,19,20]. Myostatin also negatively regulates myoblast differentiation to myotubes through the decreased expression of MyoD, myf5, myogenin and p21 through Smad 3 phosphorylation [17,21].

An important area that requires clarification is the autocrine versus the possible endocrine actions of myostatin on skeletal muscle. Although most actions of myostatin are attributable to its autocrine action, several groups [17,18••,22••,23] have shown that myostatin is present in both mouse and human serum, but the importance of circulating myostatin in relation to the autocrine actions of myostatin remain unclear.

Regulation of myostatin

With the identification of activin type II receptor B as the primary myostatin receptor and the myostatin propeptide [24,25] and follistatin as negative regulators of myostatin activity, Lee and McPherron [9] put forth the following model of myostatin regulation in 2001: the myostatin C-terminal dimer remains in a latent complex with the inhibitory propeptide. This latent complex can be further negatively regulated by binding with follistatin, and upon release of the negative regulators, myostatin is free to signal through its receptors, primarily activin type II receptor B. Recent work has sought to clarify this regulatory model, although much remains to be determined.

Hill *et al.* [22••] extended the initial regulatory model for myostatin by showing that 70% of myostatin is bound to its propeptide in serum, and that the propeptide is removed by proteolysis at the site of myostatin action. In addition, they identified a third negative regulator, the follistatin-related gene (FLRG), which appears to bind to myostatin after it has bound to its receptor and initiated signaling, thus acting as a negative feedback loop [22••]. Remarkably, Hill *et al.* [22••] could not confirm the presence of follistatin in a latent complex with myostatin in serum, although the possibility of follistatin sequestering myostatin in skeletal muscle locally was speculated. In a subsequent paper, Hill and colleagues [23] identified a fourth negative regulator of myostatin in serum, growth and differentiation factor-associated serum protein 1. Growth and differentiation factor-associated serum protein 1, a novel member of the follistatin domain proteins, appears to be highly expressed in skeletal muscle, and interacts with both mature myostatin and myostatin propeptide in normal mouse and human serum, inhibiting the biological activity of mature myostatin [23].

Two additional proteins have been identified that appear to influence the secretion of myostatin. Nicholas *et al.* [26•] showed that Titin-cap (telethonin), by binding to mature myostatin in the cytoplasm or Golgi, limited the secretion of myostatin by preventing the formation of the latent complex between mature myostatin and its propeptide. Myoblasts overexpressing Titin-cap showed increased proliferation, probably as a result of diminished myostatin secretion [26•]. Interestingly, the lack of functional Titin-cap protein is responsible for a rare limb girdle muscular dystrophy (2G) [27], and the authors speculated that the disorder might actually be the result of altered myostatin secretion [26•]. Wang and colleagues [28] reported that human small glutamine-rich tetratricopeptide repeat-containing protein (hSGT) physically interacts with mature myostatin and co-precipitates with myostatin in extracts of human muscle. The authors speculated that hSGT may act as a molecular chaperone for myostatin, but this remains to be verified [28].

Although additional research is needed to understand fully the complex, multi-component system of myostatin regulation, the identification of several myostatin regulatory proteins provides optimism that a number of strategies are possible for targeting the negative influence of myostatin in muscle-wasting disorders.

Actions of myostatin

That myostatin is an important negative regulator of skeletal muscle is clear, but details about the actions of myostatin are uncertain and inconsistencies remain. Several reports in animal models have demonstrated losses of muscle mass resulting from either elevated myostatin messenger RNA or protein, or direct myostatin administration [18••,29–32]. Reports have also shown improvements in muscle mass associated with reductions in myostatin expression or in myostatin knockouts [6,30,33,34]. Similar to the findings in animal models, increased myostatin immunoreactivity or expression has been observed in HIV-infected men with muscle wasting [35], after prolonged bed rest in young men [36], after disuse atrophy in older patients [37], and in older men and women with muscle wasting [38]. Decreased myostatin expression has been observed after strength training to elicit increases in muscle mass in young and older men and women [39]. Welle *et al.* [40] did not observe increased myostatin expression in the skeletal muscle from older compared with younger men, although the older men were healthy and myostatin protein was not measured.

In contrast to the generally consistent findings described above, recent studies have reported inconsistent patterns of myostatin expression in relation to models of rapid muscle wasting. For example, during denervation-induced atrophy in rats, myostatin expression was not increased until several days after muscle atrophy was apparent [32]. Similarly, after hindlimb suspension, myostatin-deficient mice actually lost more muscle mass than normal controls [41]. Similar results were observed in normal mice after hindlimb suspension [34]. Clarifying the basis of these different patterns of myostatin function will be important, given that many models of muscle atrophy differ vastly in the rate of tissue loss. Jeanplong *et al.* [42] showed that during underfeeding, several phases of adaptive responses occur in muscle, with specific patterns of myostatin expression that are not easily predictable. Much therefore remains to be clarified about the regulation and actions of myostatin during various phases of muscle atrophy.

With regard to the investigation of the myostatin protein, a discussion of the antibody used for protein identification is warranted. A polyclonal antibody for 'myostatin immunoreactive protein' has been developed and used to demonstrate increased levels of the protein in the muscle and serum of HIV-infected men with muscle wasting [35] and in the serum of older men and women with muscle atrophy associated with aging [38]. The specificity of this polyclonal antibody is uncertain, however, given the significant homology of GDF-11 to myostatin [1,12]. GDF-11 is also a TGF-β family member that signals through the activin type II A and B receptors and is negatively regulated by follistatin [43–45]. The development of the monoclonal antimyostatin antibody, JA16, was recently reported by Whittemore and colleagues [46••]. JA16 has been shown to be specific for myostatin, with minimal cross-reactivity with GDF-11 [22••,46••]. Experiments should be performed using both JA16 and the original polyclonal antibody [35] to validate the results of several reports using the polyclonal antibody [15,35,36,38,47•].

Myostatin as a therapeutic target

Perhaps the most important development in the past year is the solidification of myostatin as a promising therapeutic target in muscle-wasting-related disorders. A key issue for researchers had been whether myostatin plays a significant role beyond prenatal muscle development (i.e. in adult animals). To address this issue directly, Whittemore *et al.* [46••] administered the JA16 antimyostatin antibody to young (7–8-week-old), healthy mice, resulting in increased muscle strength and mass (relative to body weight) without increased organ tissue mass compared with control animals. The increases in muscle mass were seen in as little as 2–4 weeks of administration. Perhaps most importantly, the administration of JA16 to fully grown (24-week-old) mice also resulted in significant improvements in muscle mass [46••].

Similar results in different models provide additional support for myostatin as a therapeutic target for muscle wasting. For example, myostatin was identified in mouse serum and the systemic administration of myostatin inhibitors interfered with myostatin activity, slowing the loss of weight and muscle mass in myostatin overexpressing mice [18••]. Transgenic mice overexpressing follistatin exhibited muscle mass increases similar to myostatin knockout mice [9]. Using the cre-lox conditional gene targeting method to inactivate myostatin in postnatal mice, Grobet and co-workers [48•] reported significant generalized muscle hypertrophy similar to the myostatin knockout mouse, thus demonstrating that myostatin regulates muscle mass in adult animals and is a promising target for antagonist treatments designed to reverse muscle wasting.

In late 2002, two independent groups reported that inhibiting myostatin activity resulted in improved muscle function and reduced muscle degeneration in *mdx* mice, an important animal model for Duchenne muscular dystrophy (DMD) [49••,50•]. Bogdanovich *et al.* [49••] used the JA16 antibody [46••] to block the activity of myostatin *in vivo* in *mdx* mice. They reported increases in muscle mass and strength, and decreases in muscle degeneration, thus demonstrating a tantalizing alternative to the gene therapy approaches typically seen in DMD-related research. Similarly, Wagner and colleagues [50•] crossed myostatin null mice with *mdx* mice, and reported that the crosses had larger and stronger muscles with greater signs of muscle regeneration than the counterpart *mdx* mice.

The development of the JA16 antibody against myostatin, which has been used to isolate myostatin from human serum [22••], will probably speed research into the therapeutic targeting of myostatin in muscle-wasting disorders, such as DMD, sarcopenia, and cachexia. The indications from recent work [22••,46••,49••] provide strong evidence that blocking the actions of myostatin will result in improved muscle mass and strength in adult animals, which is important, because many muscle-wasting disorders with broad public health relevance are seen in adults.

Beyond the realm of public health, agriculture stands to benefit from the use of safe and inexpensive means to alter meat production and minimize the fat content in muscle through inhibiting myostatin activity, attributes that are well documented in cattle with myostatin gene mutations [2–5,10•]. A large fraction of the research on myostatin is targeted to animal science and agriculture

journals. On a darker note, substances that inhibit the actions of myostatin will certainly be exploited for sports performance improvements, and some websites already market products (with little or no scientific backing) that claim to block myostatin activity and elicit muscle growth and improved performance. As events with the recently banned synthetic steroid tetrahydrogestrinone have shown, economic forces will almost certainly result in concealed attempts to inhibit myostatin for performance gains in athletes.

Genetics of myostatin

Despite the remarkable influence of myostatin on skeletal muscle and the well-established effect of myostatin gene mutations in double-muscled cattle breeds, studies of genetic variation in humans have shown little that can be called remarkable. Ferrell and coworkers [51] identified several polymorphisms in the human myostatin gene, including amino acid-changing missense alleles, but in follow-up work by that group and others only minor associations have been observed for the most common of these polymorphisms with muscle mass or strength [51–54]. The strong role for myostatin in both muscle development and the maintenance of muscle mass in adults provides a rationale for continuing to address whether genetic variations in myostatin and members of its regulatory pathway (e.g. follistatin, FLRG) interact to influence muscle phenotypes, but the current state of the literature indicates that such work should focus on other members of the regulatory pathways in combination with myostatin. In support of this conclusion, Kocamis and Killefer [10•] pointed out that several cattle breeds exhibit myostatin gene mutations without a true double-muscled phenotype, suggesting that the double-muscled phenotype may not be explained completely by variation in a single gene. Such genetic work may prove important for identifying individuals susceptible to accelerated losses of muscle mass with aging, or alternatively, individuals with accelerated adaptive responses of muscle mass to resistive exercise.

Conclusion

Although much remains to be clarified about myostatin in skeletal muscle development and disease, the potential of myostatin and its regulatory proteins as therapeutic targets for a variety of muscle-wasting-related disorders is significant. Whereas other regulatory targets have recently been identified for skeletal muscle that may prove equally promising, myostatin is solidly established as a key target for continued study as we attempt to identify clinically useful therapies for aging and disease-related muscle wasting.

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