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Invited critical review

Myostatin — From the Mighty Mouse to cardiovascular disease and cachexia

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ABSTRACT

In 1997, McPherron et al. created the so-called Mighty Mouse: owing to the knock-out of a new member of the TGF-β superfamily of peptides, this mouse line was extremely hypermuscular and also characterized by very low body fat. The new peptide, a powerful negative muscle regulator, was named myostatin.
Apart from regulating skeletal muscle growth, myostatin has recently been reported to be significantly involved in different cardio-vascular and metabolic pathologies. This review is focused on these non-muscular myostatin actions. First, myostatin is intricately involved in regulating metabolism: it causes insulin resistance, and the advantageous metabolic profile achieved by myostatin inhibition is mainly attributable to its effects on skeletal muscle.
Myostatin is further expressed in myocardium where it exerts anti-hypertrophic, but pro-fibrotic effects. Circulating and local myostatin is elevated in chronic heart failure and poses a major player in cardiac cachexia.
Eventually, the current body of evidence regarding myostatin's significant involvement in different entities of the cachexia syndrome is summarized. Activin type-2 receptor antagonism and/or inhibitory myostatin antibodies have emerged as a promising therapeutic approach to treat the cachexia syndrome although the general applicability of this therapeutic approach to the human clinical situation has still to be demonstrated.

Contents

Abbreviations: TGF-β, transforming growth factor-β; MSTN, myostatin gene; LAP, latency-associated peptide; FLRG, follistatin-related gene peptide; FSTL-3, follistatin like 3; GASP, growth/differentiation factor-associated serum protein; LTBP, latent TGF-β-binding protein; hSGT, human small glutamine-rich tetratricopeptide repeat-containing protein; GDF, growth differentiation factor; BMP-1/TLD, bone morphogenetic protein-1/tolloid; TLL, tolloid-like; NF- B, nuclear factor- B; TNF-α, tumor necrosis factor-α; FoxO, forkhead box O; IGF-1, insulin-like growth factor-1; MEF-2, myocyte enhance factor-2; ERK, extracellular signal-regulated kinase; BMP, bone morphogenetic protein; TGFRII, TGF receptor type II; ALK5, activin receptor-like kinase-5; ActRIIB, activin receptor IIB; ActRIIB/Fc, soluble form of ActRIIB; PI3K, phosphatidylinositol-3 kinase; Akt, protein kinase B; CHF, chronic systolic heart failure; GSK-3β, glycogen synthase kinase-3; mTOR, molecular target of rapamycin; p70 S6K, p70 S6 kinase; COPD, chronic obstructive pulmonary disease.

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1. Introduction

In 1997, McPherron et al. [1] created the so-called Mighty Mouse: owing to the knock-out of a new member of the TGF-β superfamily of peptides, this mouse line was extremely hypermuscular and also characterized by very low body fat. The new peptide, a powerful negative muscle regulator, was named myostatin. The discovery of myostatin elucidated several naturally occurring "double-muscling" phenotypes of great agricultural interest, e. g., the Piedmontese, Asturiana, Marchigiana, and Belgian Blue cattle breeds [2–5].

Since then, this outstanding discovery has ignited enormous scientific and economic interest, for obvious reasons: In man, the development of myostatin-inactivating therapies holds great promise to patients suffering from cachexia, muscle dystrophy, or trauma [6,7]. At the same time, gene therapy that knocks down myostatin is clearly alluring to professional athletes seeking to break the natural limits of human physiology [8].

Apart from regulating skeletal muscle growth, myostatin has recently been reported to be significantly involved in different cardio-vascular and metabolic pathologies, such as heart failure [9,10], insulin resistance [11], and cachexia [12,13]. On these grounds, this review will focus on myostatin's impact on cardio-vascular and metabolic diseases, with particular attention paid to its potential role in cachexia. For a broader view on the diverse biological actions of this intriguing peptide, the reader is referred to several excellent reviews [14–16].

2. Gene expression, secretion, and regulation of myostatin

Myostatin, also known as GDF-8, belongs to the TGF-β protein family [1] and consists, in its full-length prepro-myostatin form, of 375 amino acids in humans [17]. Prepro-myostatin is composed of an N-terminal signal peptide (23 aa, theoretical molecular weight 2.6 kDa) followed by the pro-peptide domain (aa 24-266, 27.7 kDa) and the C-terminal mature domain (aa 267-375, 12.4 kDa) [17]. Myostatin is expressed during embryogenesis by cells of the myotome compartment of developing somites and continues to be expressed by cells of the muscle lineage throughout development as well as in adulthood [15].

The myostatin gene, MSTN, has been mapped in several vertebrate species [2,3,18–24]; in humans, it is located on chromosome 2 [17]. The fish MSTN-1 and -2 genes have also been cloned in zebrafish [25,26]. In all vertebrates, the MSTN genes are organized in three exons separated by two introns, and mature myostatin, the C-terminal active form, is encoded by the third exon [17,21]. Among the other members of the TGF-β superfamily, GDF-11 exhibits the highest sequence homology to myostatin, with 90% identity of the C-terminal region [15].

The myostatin peptide is remarkably conserved among species: regarding the N-terminal pro-peptide, mouse shows 96 and 99% homology to human and rat, respectively; the sequence of the mature C-terminal myostatin is completely conserved [27].

Proteolytic processing [15] includes the removal of the signal peptide and then, at a furin RXXR site, the cleavage of the N-terminal pro-domain from the C-terminal mature domain. The latter dimerizes by forming a disulfide bridge. Similar to the processing of other members of the TGF-β family, this step still does not create an active peptide since the dimerized mature myostatin is kept inactive by being non-covalently bound to the LAP, i. e., the N-terminal pro-domain, thereby forming the so-called small latent complex [28–30]. Moreover, there are additional inhibitors that act by binding mature myostatin: follistatin [29]; FLRG, also known as FSTL-3 [28]; GASP-1 [31], and GASP-2 [32]. Thus, follistatin, FSTL-3, and GASP-1/2 act to prevent the receptor binding of circulating mature myostatin. Decorin, a matrix-associated small proteoglycan, also binds myostatin which likewise prevents the interaction of the peptide with its receptor [33].

In addition, some proteins affect earlier stages of myostatin secretion: T-cap [34], actually a sarcomeric protein that binds the N-terminal domain of titin, binds myostatin in the golgi apparatus which prevents its secretion. As also shown for other members of the TGF-β superfamily, unprocessed extra-cellular pro-myostatin is bound to an LTBP, in this case LTBP-3, forming the large latent complex and leading to extracellular sequestration and prevention of furin cleavage [35]. Human SGT was found to bind the N-terminal signal peptide of prepro-myostatin in vitro and was therefore hypothesized to play a role in myostatin folding and/or chaperoning [36].

Hypermuscular mouse phenotypes reminiscent of the myostatin KO mouse have been generated by different modes of overexpression or direct administration of myostatin pro-peptide [37,38], follistatin [29,39], FSTL-3 [39,40], GASP-1 [39], and LTB3 [35] which supports the potential in-vivo relevance of all of these inhibitory peptides/proteins.

Eventually, the mature C-terminal dimer has to be set free from the latent complexes to bind its receptor. Notably in this context, latent myostatin is the major circulating form of the peptide, at least in mice [28,41]. For mice, it has been shown by Lee's group that the BMP-1/TLD family of metalloproteases is critically involved in this process [38,42]. Enzymes of this BMP-1/TLD family, i. e., BMP-1, TLD, TLL-1, and TLL-2, cleave the pro-peptide at the aspartate 76 residue which then abrogates the inhibitory interaction with the C-terminal peptide. BMP-1 and TLL-1 expression is detected in different skeletal muscles in the mouse [43], and BMP-1 is also found in human myocardium [9].

In summary, different inhibitors and enzymes control secretion, post-translational processing, and receptor binding of myostatin which constitutes a complex and highly dynamic network with multiple regulation sites. The mere fact that unprocessed pro-myostatin appears extracellularly, but locally in skeletal muscle [35,44] may suggest that myostatin processing by furin represents a key regulatory step with regard to its bioactivity [41,45]. Additionally, systemic circulating myostatin appears to be "one step ahead": it is prevailingly processed, but still inactivated peptide (see latent complex), the activation of which requires the action of the BMP-1/TLD enzymes.

As to the regulation of myostatin gene expression, myostatin is most abundantly expressed in skeletal muscle and, to a lower extent, in adipose tissue [1]. In rat fast muscle fibers with predominant glycolytic metabolism, it is more highly expressed than in slow muscle fibers with predominant oxidative metabolism [46]. At a much lower level, cardiomyocytes also express myostatin [47].

The "myostatin paradox" describes a positive correlation between skeletal muscle mass and both local and circulating myostatin levels, which is, at first sight, somewhat counter-intuitive for a negative muscle regulator [48]. In contrast, endurance (aerobic) exercise and most of the resistance exercise paradigms reduce myostatin expression in skeletal muscles of rat and man [46,48–50]. In differentiated mouse myoblast cells (line C2C12), TNF-α induces the expression of myostatin via the p38 MAP kinase and NF- B [13]. In the same cell line, myostatin promoter activity is up-regulated by the transcription factors Smad-2/-3 and -4, posing the link to TGF-β signaling, as well as FoxO-1 [51]. Glucocorticoids up-regulate myostatin expression: In the human myostatin gene, glucocorticoid response elements have been identified [52], and the dexamethasone-related increase in myostatin promoter activity has been shown in vitro in mouse C2C12 and in rat L6 skeletal muscle cells [52,53] as well as in vivo after dexamethasone injections in rats [54]. In terms of post-translational mechanisms, in-vitro experiments in C2C12 cells indicate that glucocorticoid-induced down-regulation of microRNA 27, which binds to the 3′-untranslated region of myostatin mRNA and expedites its degradation, may contribute to enhanced myostatin expression [55].

With regard to myocardial tissue [56], IGF-1 has been shown to mediate the cyclic stretch-induced up-regulation of myostatin expression in neonatal rat cardiomyocytes, via p38 kinase and the transcription factor MEF-2. Angiotensin-II, a major player in cardiac hypertrophy and remodeling, up-regulates myostatin expression in cultured rat neonatal cardiomyocytes via the angiotensin type-1 receptor, the p38 MAP kinase and MEF-2 [57]. In adult rat cardiomyocytes, urotensin-II and urocortin, two hypertrophy-inducing peptides, stimulate the