REVIEW OPEN ACCESS

Myostatin: Functional Divergence, Evolutionary Insights, and Therapeutic Potential

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ABSTRACT

The transforming growth factor β (TGFβ) family members, growth differentiation factor 11 (GDF11) and myostatin (MSTN), share 89% sequence identity in their mature forms but exhibit distinct biological functions. While MSTN is a well-established negative regulator of skeletal muscle growth, the role of GDF11—particularly in postnatal development—remains controversial. This controversy stems in part from the perinatal lethality of Gdf11-null mice, which has led to reliance on recombinant proteins that may not fully replicate endogenous GDF11 activity. In contrast, genetic studies using knockout or conditional knockout models consistently suggest that GDF11 and MSTN play opposing roles in tissue development and homeostasis. This review explores the evolutionary divergence of GDF11 and MSTN, highlighting their distinct expression patterns and functions across species. We discuss their proteolytic processing, signaling mechanisms, and physiological roles in development, adulthood, and aging. Additionally, we evaluate the therapeutic potential of recombinant GDF11 and the implications of MSTN inhibition. Notably, while the mature domains of GDF11 and MSTN are highly conserved, their prodomains exhibit significant divergence, suggesting unique regulatory mechanisms for GDF11. This comprehensive analysis aims to clarify the functional distinctions between GDF11 and MSTN and their relevance in health and disease.

Keywords: GDF11, myostatin, TGFβ family, skeletal muscle, development, aging, proteolytic processing, evolutionary divergence.

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Cytokines of the transforming growth factor β (TGFβ) family, including activins, growth differentiation factors (GDFs), bone morphogenetic proteins (BMPs), and TGFβs, have been extensively implicated in the regulation of developmental

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patterning, cellular proliferation and differentiation, and the maintenance of tissue home ostasis (Morikawa, Derynck, and Miyazono 2016). Among the TGFβ family members, there are two highly homologous protein, myostatin (MSTN), which share 89% sequence identity in their mature form but exhibit distinct endogenous functions. While Gdf11 is expressed broadly in numerous tissues, Mstn is expressed primarily in skeletal muscle (Gamer et al. 1999; McPherron, Lawler, and Lee 1997; Nakashima et al. 1999). The functional divergence of GDF11 and MSTN is indicated by the fact that their mutation in animals leads to the development of largely dissimilar features. For instance, while the genetic deficiency of MSTN leads to a hyper muscular phenotype in various species (McPherron, Lawler, and Lee 1997; McPherron and Lee 1997; Schuelke et al. 2004), homozygous deletion of Gdf11 generates defects in axial skeletal pat terning and organ development in mice (McPherron, Lawler, and Lee 1999). However, unlike the relatively consistent reports of the function of MSTN in suppressing skeletal muscle growth, the reports of GDF11 function, particularly those examining the post natal role of GDF11, remain highly controversial. One of the main reasons for this controversy lies in the fact that Gdf11-null mice, unlike Mstn-null mice, show perinatal lethality (McPherron, Lawler, and Lee 1999), leading most studies to utilize recombinant proteins that cannot fully recapitulate the complex endogenous functions of GDF11. Importantly, in contrast to studies that utilized recombinant GDF11 or MSTN proteins, those that applied genetic knockdown, knock out, or conditional knockout techniques revealed rela tively unvarying results despite their being fewer in number, and most have reported the positive roles of GDF11 and the negative roles of MSTN in the regulation of the development of various tissues. In this review, we first present the similarities and differences between GDF11 and MSTN from an evolutionary point of view and summarize the insights obtained to date regarding the biological processing, signaling mechanisms, and physio logical functions of GDF11 and MSTN during develop ment, adulthood, and aging. We also discuss the potential of recombinant GDF11 protein as a therapeutic option for various clinical conditions and the possible adverse effects of GDF11 inhibition mediated by MSTN inhibitors. Evolution and biology of GDF11 and MSTN Evolutionary analysis of GDF11 and MSTN The remarkable sequence similarity between GDF11 and MSTN has led to the assumption that they were derived from the same ancestral gene through gene duplication. Indeed, analysis of multiple invertebrate species revealed that they harbor a single homologous gene corresponding to GDF11 and MSTN (Funkenstein and Olekh 2010). For instance, in Caenorhabditis elegans, daf-7 was shown to encode a homolog of GDF11 and MSTN, while in fruit flies (Dro sophila melanogaster), myoglianin (Myo) was found to exhibit the highest sequence homology to GDF11 and MSTN (Fletcher and Kim 2017; Funkenstein and Olekh 2010; Nagata et al. 2020). An important question that arose from these identifications was whether the divergence of

GDF11 and MSTN occurred at the time of the emergence of verte brates. To provide an explanation, a phylogenetic study was conducted in various invertebrate and vertebrate species, and importantly, the amphioxus (Branchiostoma belcheri) (Xing et al. 2007), which is an invertebrate known to be the closest relative of the vertebrates, was included in the analysis. Additionally, the amino acid sequences of the full-length protein, the propeptide with the signal peptide, and C-terminal peptide were separately compared. All phylogenetic trees demon strated a clear separation between the GDF11 and MSTN clusters that appeared after the divergence of vertebrates from the amphioxus, confirming that the gene duplication event occurred at the time when vertebrates and inver tebrates split. Notably, unlike the single isoform of the MSTN gene observed in mammals, two isoforms of the mstn gene have been detected in fish (Funkenstein and Olekh 2010). The reason for and functional significance of the diver gence of the two mstn genes in fish remains to be clarified. Interestingly, many of the reported functions of the invertebrate MSTN/GDF11 protein are very different from the well-established suppressive role of vertebrate MSTN in the development of multiple tissues, and the broad expression pattern of the ancestral protein more closely resembles the expression pattern of vertebrate GDF11 (De Santis et al. 2011; Demontis et al. 2014; Fletcher and Kim 2017; Nagata et al. 2020). These observations imply that MSTN most likely emerged from the ancestral gene to allow more specific control of skeletal muscle growth in vertebrates, although the relatively small amount of information available on the function of invertebrate MSTN/ GDF11 limits further interpretation (De Santis et al. 2011; Demontis et al. 2014; Greer et al. 2008). The reportedphy siological roles of the ancestral protein in invertebrates will be discussed in more detail later. Proteolytic processing of GDF11 and MSTN Both GDF11 and MSTN, like the other members of the TGF-β family, are initially synthesized as precursor proteins and are subsequently cleaved by proteases to produce biologically active mature ligands (De Santis et al. 2011; Walker et al. 2016; Xing et al. 2007). More specifically, following the removal of the signal peptides by signal peptidases, furin-like proteases recognize and cleave the conserved RSRR residues of GDF11 and MSTN, gen erating N-terminal propeptides and C-terminal mature peptides (Gunther, Georgi, and Riddle 2000; Walker et al. 2016). The proprotein convertase PC5/6 was demonstrated to spe cifically cleave GDF11 by recognizing the RSRR↓N clea vage motif, which is not present in MSTN (Essalmani et al. 2008; Walker et al. 2016). Accordingly, mice deficient in PC5/6 were shown to phenocopy Gdf11 null mice by exhibiting anterior homeotic transformations of the vertebrae, the lack of a tail, kidney agenesis, and retarded ossification (Essalmani et al. 2008). After the cleavage of the RSRR site by a furin-like protease, the propeptide and mature pep tide remain noncovalently associated with each other, forming a latent complex that is unable to bind receptors. However, a recent study showed that the latent MSTN complex can also become

capable of binding receptors after being exposed to acidic conditions. Exposure to acidic conditions led to a conformational change of the latent MSTN complex and stimulated it to become in a triggered state, in which the pro- and mature domains still remain associated but were capable of signaling (Chang et al. 2018). The fact that MSTN can exist in both fully latent and triggered states further demonstrates the complexity of its activa tion mechanism. Nonetheless, to achieve full signaling activity, both the latent GDF11 and MSTN complexes require additional cleavage of the N-terminal propeptides by BMP1/tolloid (TLD)-like metalloproteinases, which dissociate the propeptides from the mature C-terminal dimers, thus freeing the ligands for receptor binding (Essalmani et al. 2008). Mature dimers can also be inhibited by the addition of propeptides both in vitro and in vivo23. To examine the rates of the evolutionary changes of the residues of GDF11 and MSTN, we utilized a recently developed webtool, Aminode (Chang et al. 2018), and analyzed the evolu tionarily constrained regions (ECRs) of the proteins. As expected, the mature domains of GDF11, MSTN, activins, and TGF-βs were remarkably well-conserved among vertebrate spe cies, displaying extremely low rates of amino acid sub stitution in most positions. Surprisingly, only GDF11 presented a striking degree of sequence con servation in the prodomain, emphasizing the functional significance of this region. In fact, while GDF11 and MSTN share 89% amino acid sequence identity in their mature domains, which differ by only 11 residues, their prodomains share only 48% amino acid sequence identity. This suggests the strong possibility that GDF11 prodomains may be associated with distinct and crucial extracellular regulatory mechanisms and biological functions that are not observed for the prodomains of MSTN, which warrants further investigation that may uncover significant differences that were previously unnoticed for the mature ligands (Essalmani et al. 2008; Walker et al. 2016).

Conclusion and Future Perspectives

GDF11 and MSTN, despite their high sequence similarity, exhibit distinct biological roles, with MSTN primarily acting as a negative regulator of muscle growth and GDF11 playing broader roles in development, tissue homeostasis, and aging. While the function of MSTN is well-established, the physiological and therapeutic roles of GDF11 remain controversial, largely due to conflicting results from recombinant protein studies versus genetic models. Evolutionary analyses suggest that GDF11 and MSTN diverged from a common ancestral gene in vertebrates, with GDF11 retaining a more widespread expression pattern akin to invertebrate homologs, whereas MSTN evolved to specialize in muscle growth regulation. Proteolytic processing and activation mechanisms further differentiate these two ligands, with GDF11's prodomain displaying unusually high conservation, implying unique regulatory functions. The discovery of latent and

"triggered" states of MSTN adds complexity to its activation, suggesting that similar mechanisms may exist for GDF11 but remain unexplored. Additionally, the differential effects of GDF11 and MSTN in aging and disease highlight their potential as therapeutic targets—GDF11 for regenerative medicine and MSTN inhibition for muscle-wasting disorders. Further investigation into the functional significance of GDF11's highly conserved prodomain could reveal novel regulatory mechanisms distinct from MSTN. Studies should explore whether GDF11, like MSTN, can exist in a "triggered" latent state and how extracellular conditions influence its activation. The perinatal lethality of Gdf11-null mice complicates postnatal studies; thus, inducible and tissue-specific knockout models should be prioritized to dissect GDF11's roles in adulthood and aging. Humanized mouse models or organoid systems may help bridge the gap between animal studies and potential clinical applications. The conflicting effects of recombinant GDF11 in different studies suggest that dosing, timing, and tissue specificity are critical. Future work should optimize delivery methods to mimic endogenous GDF11 activity. Given that MSTN inhibitors (e.g., antibodies, gene therapy) are in clinical trials for muscle atrophy, their potential cross-reactivity with GDF11 and off-target effects on non-muscle tissues must be carefully evaluated. Studying GDF11/MSTN homologs in basal vertebrates and invertebrates could provide deeper insights into how their functional divergence arose. The existence of two mstn genes in fish warrants investigation into whether they have subfunctionalized roles in muscle versus non-muscle tissues. If GDF11 proves beneficial in aging or degenerative diseases, developing targeted agonists or gene therapies to enhance its activity without disrupting MSTN signaling will be crucial. Conversely, strategies to selectively inhibit MSTN while sparing GDF11 could maximize therapeutic benefits in muscle disorders while minimizing adverse effects.

Authors Contribution

Paing Oo Kyaw; written the manuscript, Muhammad Farhab; revised the manuscript, Yu-Guo Yuan; supervised the project

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Conflict of Interest

Authors declare no conflict of interest

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