Review Article

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Myostatin: A Molecular Breeding Tool to Augment Meat Production

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Abstract

Myostatin (MSTN) is a recently identified muscle growth regulator, which belongs to decapentaplegic-Vg-related (DVR) related subfamily of transforming growth factor beta (TGF-â) superfamily.Molecular genetic studies in various species have determined that mutations in MSTN gene results in dramatic increase in muscle growth due to hyperplasia and hypertrophy. It is expressed predominantly in skeletal muscle and acts as a negative regulator of skeletal muscle growth by suppressing proliferation and differentiation of myoblasts and satellite cells in mammals. It has high affinity for the activin IIB receptor and weak affinity for activin IIA receptor. The MSTN signaling pathways have been categorized into Smad-mediated and NonSmad pathways.The present review mainly emphasizes on history, regulation and various strategies to block MSTN signaling pathway.

Keywords: Myostatin; GDF8; Activin IIB Receptor and Muscle Growth.

Introduction

Traditional breeding methods, adopted from principles of quantitative genetics, consider each individual as a black box with an infinite number of genes controlling the expression of all traits of the individual. Molecular genetics is now deciphering this black box by elucidating the effect of single genes on the phenotypic expression of traits. The initial success that had an influence on animal breeding was the discovery of genetic markers. The application of genetic markers necessitates the establishment of linkage disequilibrium phases between markers on the DNA of chromosomes and the genetic variability of traits of interest through extensive "QTL mapping" experiments (Albers *et al.,* 2006). Despite the fact that these experimental findings were superior to various other approaches, many animal breeders couldn't utilize QTL findings completely.

However, information on QTLs may be used in "candidate gene" approach which employs prior information to postulate that a particular gene (the candidate) may be accountable for a known major genetic effect. Research then, emphasized on identifying the gene through prior information on its sequence (like through cross species information, such as humans or mice), identifying variation in the sequence of the gene, and finally correlating the various alleles with phenotypic variation of the characteristic in question (Albers *et al.,* 2006). A recent example of accomplishment with this approach in vertebrates is identification of a new growth and differentiation factor named Myostatin (MSTN) gene, which has endowed new perceptions for the better understanding of the muscle growth regulation in vertebrates (McPherron and Lee, 1997).

Breakthrough Discoveries

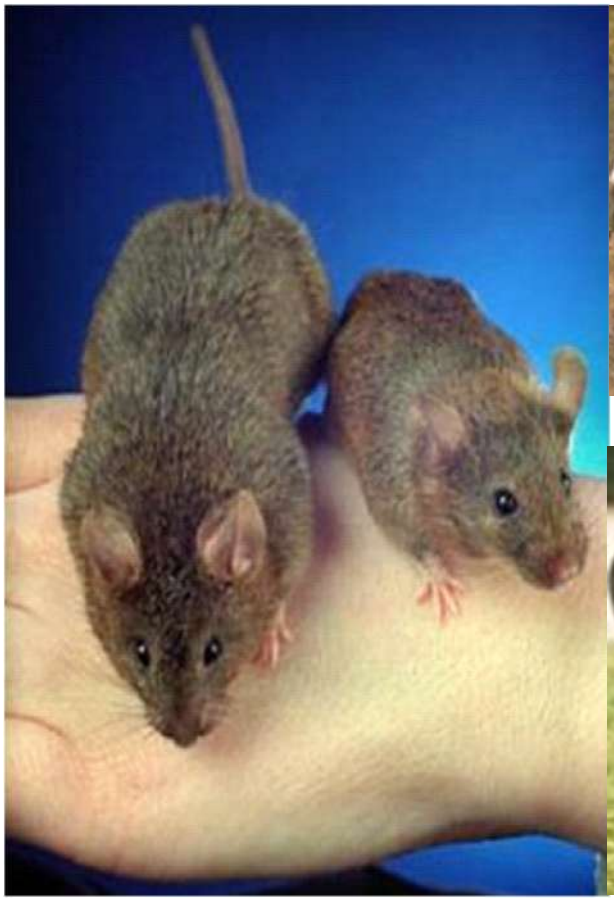
The role of MSTN in the growth of skeletal muscle was first discovered by geneticists Se-Jin Lee and Alexandra McPherron in 1997, using the technique of gene disruption in mice. Mstn null animals exhibit

an approximate doubling of skeletal muscle mass throughout the body as a result of a combination of hyperplasia and hypertrophy (Mcpherron *et al.,* 1997). The regulatory function of MSTN also found to be conserved in different species, as animals with genetic mutations in the MSTN gene, seen in mouse (Fig 1A), Belgian Blue cattle (Fig 1B) and the whippet dog (Fig 1C) all exhibiting hyper muscled phenotype, and this has also been documented in human beings (Schuelke *et al.,* 2004). Certainly, sequencing the MTSN gene in Belgian Blue and Piedmontese double-muscled animals revealed two mutations, which were predicted to be highly missense. A frame shifting 11-bp deletion in the third exon, truncating major portion of the bioactive MSTN carboxyterminal domain (Belgian Blue cattle). The substitution of the fifth of nine highly conserved carboxyterminal cysteines mediating an intramolecular disulfide bridge stabilizing the structure of the bioactive domain in Piedmontese cattle (Kambadur *et al.,* 1997: McPherron and Lee, 1997). In addition to these, Clop *et al.,*(2006) reported that Texel sheep were also double muscled due to a mutation 3' UTR of the myostatin gene, which creates target sites for the microRNAs miR-1 and miR-206.The discovery of all these findings suggested that myostatin play as an important role in regulation of muscle growth.

Fig. 1: Myostatin Null Animals

Fig. 1a: *Myostatin knock-out mice* (McPherron *et al.,* 1997: Lee *et al.*, 1999: Amthor *et al.*, 2007).

Fig. 1b: *Double-muscled cattle* (McPherron *et al.,* 1997: Lee *et al.*, 1999).



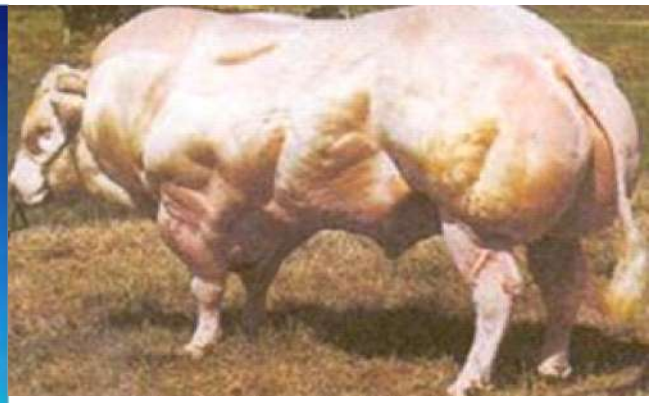


Fig. 1c: *Whippets* (Mosher *et al.*, 2007).

Myostatin/Growth differentiation factor- 8 (GDF- 8)

Myostatin (MSTN), also known as growth differentiation factor- 8 (GDF- 8) is a member of the decapentaplegic-Vg-related (DVR) related subfamily belonging to transforming growth factor beta (TGF-â) superfamily. A full-length sequence of myostatin cDNA was obtained from a murine skeletal muscle library. The myostatin protein sequence deduced contains all the characteristic of TGF-â superfamily members, besides a signal sequence for secretion, a proteolytic processing site, and a carboxy-terminal region containing the conserved pattern of nine cysteine residues (Mcpherron *et al.,* 1997). Parallel to other members of TGF-â superfamily, myostatin is also synthesized as a 376 amino acid precursor protein comprising of a signal sequence, an Nterminal propeptide domain and a C-terminal domain that gives rise to the active ligand (Fig. 1).

Myostatin activation necessitates cleavages of the precursor protein by two proteolytic enzymes family. The ûrst cleavage by furin family enzymes removes the 24-amino acid signal peptide (Lee, 2004). The second cleavage by BMP1/Tolloid matrix metalloproteinase occurs at an RSRR (Arg-Ser-Arg-Arg) site at amino acids 240–243 numbered from the ûrst amino acid following the signal sequence and leaves N-terminal and C-terminal domains of 27,640 Da and 12,400 Da, respectively (Lee, 2004). Mature myostatin is a disulûde-linked dimer of C-terminal domain and is 100% identical among human, mouse, rat, pig, chicken, turkey, and dog.

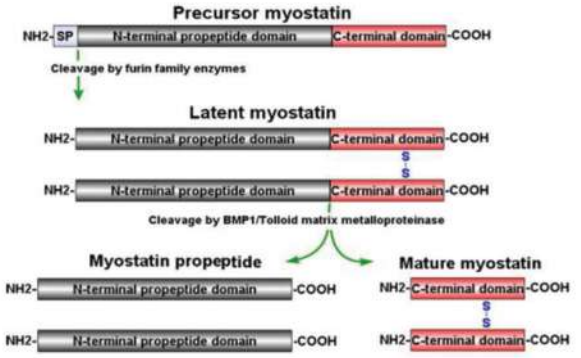
MSTN is expressed initially in the myotome compartment of developing somites and continues to be expressed in the myogenic lineage throughout embryonic development and in adult animals. It is expressed predominantly in skeletal muscle and functions as a negative regulator of skeletal muscle growth by suppressing proliferation and differentiation of myoblasts and satellite cells in mammals (McPherron *et al.*, 1997). MSTN is wholly expressed in skeletal muscle during embryonic myogenesis (Mcpherron *et al.*, 1997) but in adult myogenesis, it is expressed in other tissues *i.e.*, myocardial tissue, adipose tissue and mammary gland in addition to skeletal muscle (Mcpherron *et al.*, 1997; Allen *et al.*, 2008).

Initial studies have explicated that MSTN generally performs two important functions in regulating muscle growth. One major function is to regulate the number of muscle fibers that are formed during embryonic myogenesis and numerous investigations have shown that the increases in fiber number seen in MSTN null mice resulting mostly from increase in the numbers of type IIx and IIb fibers (McPherron, *et al*. 2009). A second function of MSTN is to control postnatal growth of muscle fibers, and increase in size of type II fibers has been well established in adult mice treated with MSTN inhibitors.

Myostatin signaling Pathways

MSTN is an extracellular cytokine, similar to other members of the TGF- ß family, it mediates the signal by binding a cell-bound receptor called the activin type II receptor. (Lee 2001; Mcpherron 2001). Once activated, MSTN has high affinity for the activin IIB receptor (ActRIIB) and weak affinity for activin IIA receptor (ActRIIA), both of which bind multiple ligands involved in regulation of muscle growth (Lee *et al.*, 2005). The signaling pathways for MSTN have been studied in the past several years and can be divided into Smad-mediated and Non-Smad pathways (Huang *et al.*, 2011).

Fig. 2. Proteolytic processing of myostatin protein (Huang *et al.*, 2011).



Regulation of Myostatin

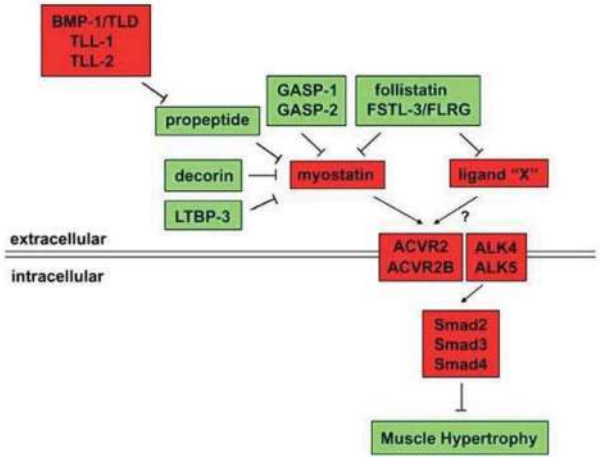
Earlier investigations by various researchers have identified several inhibitors which prevent binding of MSTN to ActRIIB. Numerous studies have provided evidence demonstrating that excess propeptide is capable of inducing muscle growth by blocking MSTN signaling rather than interfering with MSTN production (Matsakas *et al.*, 2009 and Foster *et al.*, 2009). Besides propeptide, several other proteins have been reported. One of them is follistatin, which binds MSTN and inhibits its activity by preventing its binding to the receptor (Zimmers *et al.*, 2002). Follistatin- like 3 protein (FLRG) has more than 30% homology with follistatin and was also shown to bind circulating MSTN. Another inhibitor of MSTN is growth and differentiation factor- associated serum protein 1 (GASP-1). It was found that GASP- 1 also bind to the MSTN propeptide and probably regulate the activation of MSTN through proteolytic cleavage (Hill *et al.*, 2002). Furthermore, MSTN can be inactivated upon covalent binding to latent TGF- ß binding protein 3 (LTBP 3). This inactive complex of MSTN/LTBP3 is utilized for storage of MSTN in the extracellular matrix (Anderson *et al.*, 2008).

Molecular approaches for blocking Myostatin activity

Owing to distinct tissue-specific function, MSTN is an important target for the improvement of therapies for chronic muscle degeneration (such as sarcopenia or muscle degenerative diseases), acute muscle loss (such as cachexia), and even metabolic diseases (such as obesity and Type I diabetes). Various pharmacological strategies for the down regulation of the MSTN pathway were being explored. These include direct administration of (a) chemical substances such as the SB-431542 selective ALK5 antagonist (Watt *et al.*, 2010) or follistatin-inducing deacetylase inhibitors (Minetti*et al.*, 2006) (b) blocking

anti-MSTN antibodies (Schuelke *et al.*, 2004) (c) the MSTN wild typeor mutated (p.D76A) propeptide (Bogdanovich *et al.*, 2005), (d) soluble forms of the ACVR2B receptor (Lee *et al.*, 2005), or (e) antisense or small interfering RNAs directed against MSTN (Tripathia *et al.*, 2012).

Fig. 3: Regulation of Myostatin signaling pathway and Muscle growth (Lee, 2010)



Gene therapies like injection of naked plasmid or viral vectors (particularly recombinant adenoassociated virus) were being developed with an objective to achieve longer lasting expression of MSTN inhibitors like MSTN propeptide, follistatin, FLRG and GASP-1, or small interfering RNAs targeting MSTN. Alike, MSTN vaccination intended at disrupting the immunological tolerance for this self-antigen, has been used to down regulate MSTN in mice, causing an increase in muscle growth and grip strength. Similar approaches can be implemented in livestock species to augment muscle growth and ultimately meat production. Inadequate number of experiments have been conducted in livestock with regards to MSTN inhibition, hence very few reports were available in the literature. Amongst these, Kim *et al.*, injected anti-MSTN antibodies in chicken eggs (yolk) and found an increase in body weight (4%) and muscle growth (5%) in the offspring at 35 days. Long *et al.*, immunized lots of six pigs with two doses of full-length recombinant porcine MSTN produced in Escherichia Coli. Weaned animals were immunized four times at one to two week intervals, slaughtered and analyzed at 100 kg. The authors found significant effects of MSTN immunization on carcass lean percentage (+7%) and intramuscular fat (-26%). While all these results in livestock species obviously require independent affirmation, which indicates that MSTN blockade might indeed be of useful in livestock production.

Furthermore, Lee (2005) speculated that pharmacological agents capable of blocking MSTN activity have been shown to cause significant increase in muscle growth when administered systemically to adult mice expounding that MSTN plays a significant role in regulating muscle homeostasis postnatally by suppressing muscle growth. At the same time, over expression of MSTN results in the reduction of muscle growth suggesting MSTN to be a negative regulator of skeletal muscle growth.

Conclusion

The discovery of MSTN has provided new insights pertaining to molecular genetic regulatory mechanisms underlying the muscle growth in vertebrates. Hence MSTN blockade strategies can be exploited in molecular breeding principles to improve the meat production by using techniques like transgenic engineering, pharmacological blockade or immunomodulation. Besides the imperative role of MSTN in muscle growth regulation make it as a potential candidate for marker identification in marker assisted selection schemes.

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