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PERSPECTIVES

Myostatin (GDF-8) as a Therapeutic Target for the Prevention of Osteoporotic Fractures

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Abstract

It is widely recognized that myostatin (GDF-8), a member of the transforming growth factor beta (TGFβ) superfamily of growth and differentiation factors, suppresses muscle growth and development. Increased myostatin expression can induce muscle wasting and muscle atrophy, whereas myostatin deficiency leads to a marked increase in muscle mass. Myostatin deficiency also appears to improve bone density and bone regeneration. Hence, myostatin inhibitors may not only improve muscle mass and reduce fall risk but might also increase bone strength. Recent studies have shown that myostatin exerts its effects by binding the type IIB activin receptor (ActRIIB, or Acvr2b), activating a TGFβ signaling pathway, and inhibiting Wnt signaling. Myostatin function can be inhibited by a recombinant myostatin propeptide, myostatin antibody, or soluble decoy type IIB activin receptor. Recent studies demonstrate that these myostatin inhibitors are effective at increasing both muscle mass and bone formation *in vivo*. These new findings reveal that molecules targeting myostatin signaling may be novel, effective therapeutic agents for improving muscle strength, increasing bone mass, and preventing falls and bone fractures. *IBMS BoneKEy*. 2010 January;7(1):8-17.

Keywords: Acvr2b; ActRIIB; Muscle hypertrophy; Myostatin inhibitors; Sarcopenia; Falls

Function of Myostatin (GDF-8) *In Vivo*: Evidence From a Mighty Mouse

Myostatin, also known as growth and differentiation factor-8 (GDF-8), was first identified in 1997 by Alexandra McPherron, Se-Jin Lee, and colleagues at the Johns Hopkins University School of Medicine (1). *In situ* hybridization experiments showed that the molecule was highly expressed during development in the myotome compartment of somites, and myostatin transcripts could still be detected in the skeletal muscles of adult mice. In order to better understand the function of myostatin in muscle growth and development, myostatin-deficient mice were generated. The myostatin knockout mice revealed a dramatic phenotype characterized by myofiber hypertrophy and hyperplasia resulting in a doubling of muscle mass (1). The mice not only demonstrated larger muscles but also decreased body fat (2), suggesting that myostatin might play a role in the regulation of adipogenesis and fat metabolism. Since that time, naturally occurring mutations in the myostatin gene have been identified in Belgian blue cattle (3), Texel sheep (4), whippet racing dogs (5), and in a child from Germany (6). In all of these cases, myostatin deficiency is associated with a marked increase in muscle mass. The converse also appears to be true, where treatment with recombinant myostatin causes muscle wasting (7) and transgenic overexpression of myostatin decreases muscle mass (8). Conditions associated with muscle loss, such as prolonged bed rest or disuse, exposure to microgravity, and cancer- and AIDS-related cachexia, are all associated with increased expression of myostatin and/or its receptor (9-11; Fig. 1). Together, these findings reveal that myostatin is a potent inhibitor of muscle growth, development, and hypertrophy. Myostatin loss-of-function can enhance muscle mass, whereas elevated

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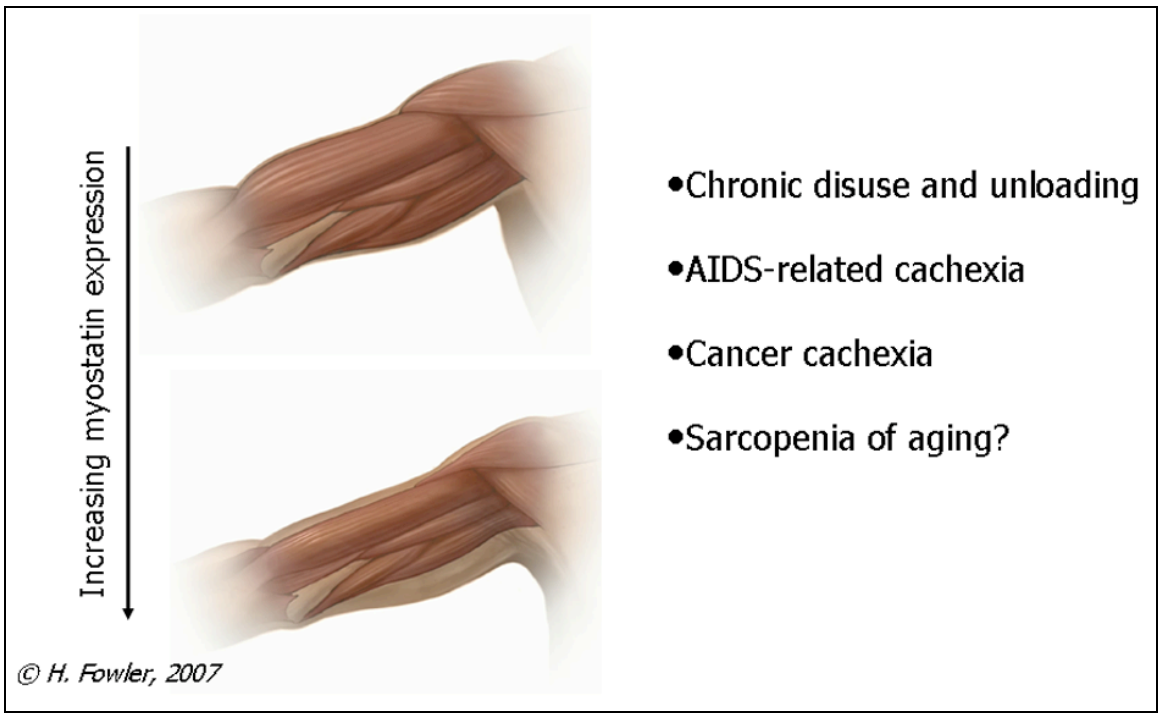

Fig. 1. Myostatin expression suppresses muscle growth, development, and hypertrophy. Elevated myostatin levels are thought to be associated with conditions that induce muscle atrophy, such as disuse, AIDS- and cancer-related cachexia, and possibly age-associated muscle wasting (sarcopenia).

myostatin expression induces muscle atrophy.

Myostatin Signals Through the Type IIB Activin Receptor (ActRIIB, or Acvr2b) to Regulate the Differentiation of Mesenchymal Stem Cells

Myostatin is similar in structure to other members of the TGFβ superfamily, particularly GDF-11 (12). Like many other TGFβ ligands myostatin is secreted in a latent form, bound to a propeptide from which it must be cleaved to generate an active ligand capable of binding its receptor, the type IIB activin receptor (ActRIIB, or Acvr2b; Fig. 2). Although myostatin is also found in serum, it circulates predominantly in a latent, inactive form (12). Hence, the primary mechanism of myostatin action is believed to be autocrine in nature (13-14). Follistatin, a factor capable of binding activin and many BMPs, can bind myostatin in skeletal muscle and inhibit its activity, whereas follistatin-related gene (*FLRG*, or *Fstl3*) binds circulating myostatin in serum (12;15). Mice overexpressing follistatin or a dominant negative form of ActRIIB show significant increases in muscle mass (16) (Fig. 2). Myostatin binding to ActRIIB forms a functional complex with the type-I receptors Alk4 or Alk5, and regulates the expression of its target genes via a TGFβ signaling pathway involving Smad2/3 (17-18). Myostatin also suppresses Wnt4 expression, inhibiting myoblast proliferation (19-21), and myostatin-induced phosphorylation of Smad3 can alter the expression of Wnt/β-catenin pathway genes (22).

Myostatin was initially identified as a factor regulating myogenic differentiation because its expression was localized to developing skeletal muscle, and because myostatin loss-of-function was observed to have dramatic effects on muscle mass in mice. It was, however, also noted that mice lacking myostatin showed decreased body fat (2). This was thought to be an indirect effect of the increased muscle mass on metabolism. Since that time, we and others have found that myostatin deficiency inhibits adipogenesis *in vivo*, even when mice are fed a high fat diet (23). Consistent with a direct effect on adipogenesis, myostatin has been observed to promote adipogenesis in multipotential mouse C3H 10T(1/2)

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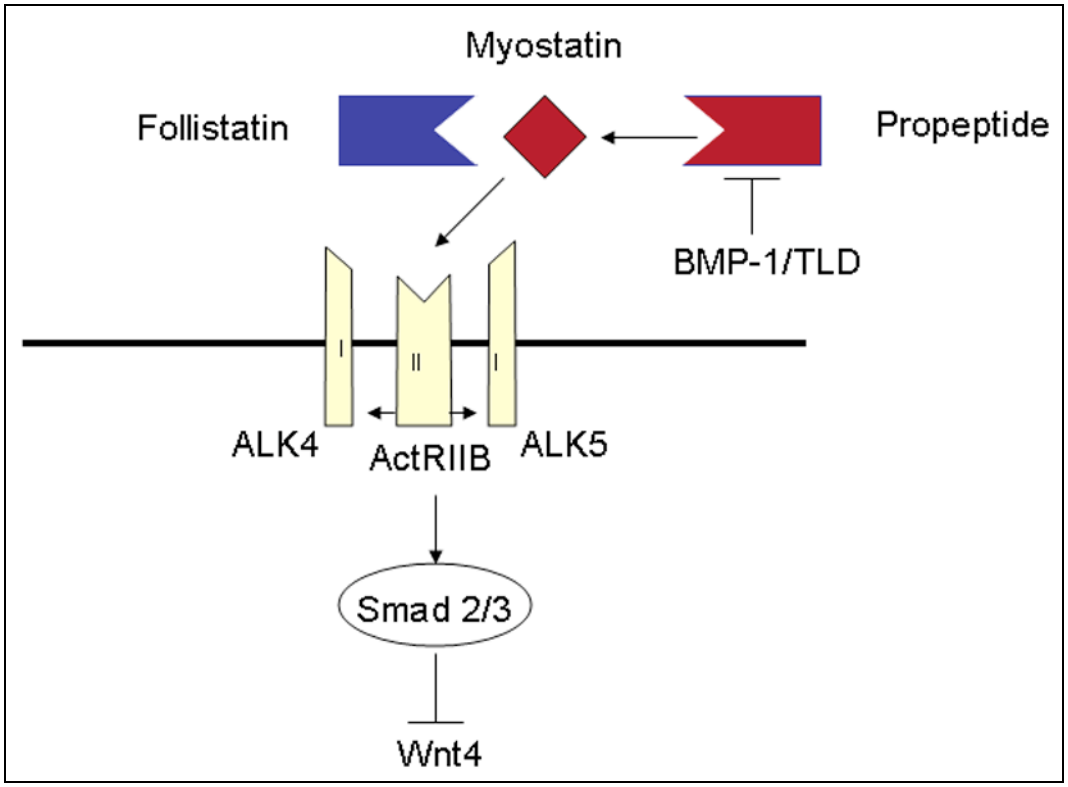

Fig. 2. Myostatin must be cleaved from a propeptide to generate an active ligand capable of binding the type IIB activin receptor (ActRIIB, or Acvr2b). The BMP-1/tolloid (TLD) metalloproteinase is required for propeptide cleavage. Myostatin and ActRIIB form a complex with type I receptors to activate a TGFβ signaling pathway involving phosphorylation of Smad 2/3. Myostatin is also known to suppress expression of Wnt4, and to alter the nuclear translocation of β-catenin, suggesting a role for myostatin in Wnt signaling.

mesenchymal stem cells (24;25). It should, however, also be noted that muscle-specific overexpression of a dominant-negative myostatin receptor reduces fat mass whereas adipocyte-specific overexpression of the dominant negative receptor does not alter adiposity (26), suggesting that there is an indirect effect of double-muscling on fat mass. Although certain *in vitro* studies have indicated that myostatin can inhibit adipocyte differentiation in 3T3-L1 mouse preadipocytes (27;28) and human bone marrow stromal cells (22), transgenic overexpression of the myostatin propeptide, which inhibits myostatin binding, also decreases adipogenesis (29). These data are consistent with our own observations of bone marrow-derived stromal cells (BMSCs) isolated from normal and myostatin-deficient mice cultured in adipogenic and osteogenic medium (30). BMSCs from mice lacking myostatin showed greater staining for alkaline phosphase and increased numbers of mineralized nodules than BMSCs from normal mice, whereas cells from wild-type mice cultured in adipogenic medium showed a greater number of Oil Red O positive adipocytes than cells from myostatin knockout animals. These data suggest that myostatin deficiency may directly enhance the osteogenic potential of bone marrow progenitors (30) (Fig. 3A).

Myostatin Plays a Role in Bone Metabolism and Bone Regeneration

The *in vitro* evidence reviewed above showing that myostatin deficiency has a direct, positive effect on osteogenesis is further supported by *in vivo* studies on bone formation and bone density. Mice lacking myostatin have increased bone mineral density (BMD) in the limb skeleton (31-32), spine (33), and jaw (34), and the increased whole-body BMD and bone mineral content found in myostatin knockout mice persists into old age (35). These data are further supported by genetic studies in human

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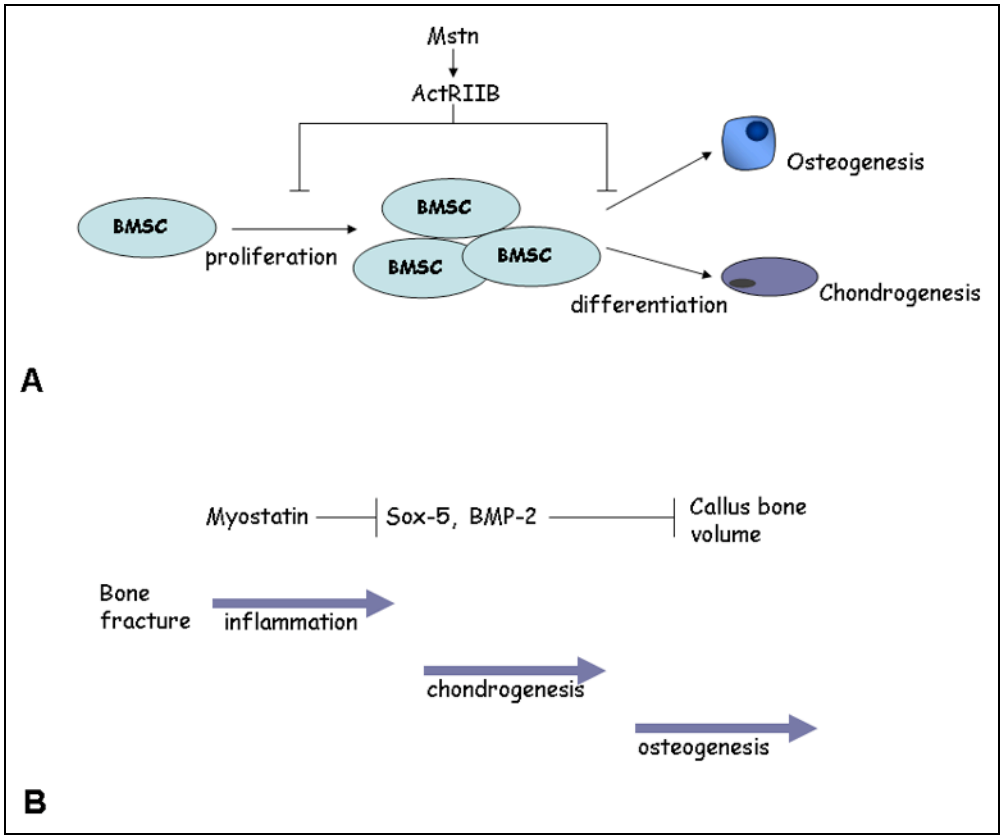

Fig. 3. A. Myostatin binding to ActRIIB in mesenchymal stem cells is hypothesized to directly antagonize osteogenic and chondrogenic differentiation, as well as the proliferation of BMSCs. B. Myostatin is highly expressed in the fracture callus immediately (24 hours) following injury, and myostatin deficiency increases expression of Sox-5 and BMP-2, suggesting that myostatin expression limits the number of progenitor cells and ultimately the size and bone volume of the fracture callus.

populations showing that myostatin gene polymorphisms are associated with variation in peak BMD (36). Furthermore, inhibition of normal myostatin signaling by transgenic overexpression of myostatin propeptide increases BMD in mice (37). Although the mechanism(s) by which myostatin regulates bone formation and density is not yet well-understood, there is also evidence that myostatin plays an important role in fracture healing and bone repair. The morphological and molecular events involved in fracture healing are relatively well-understood and can be separated into three general phases: an initial inflammatory phase, a chondrogenic phase, and an osteogenic phase (38-40) (Fig. 3B). Myostatin expression in the fracture callus has been detected during the inflammatory phase (38), but its role was difficult to interpret since this factor was most well-known for its effects on muscle. Experiments using an open fracture model in myostatin-deficient mice demonstrated that an absence of myostatin signaling increased fracture callus size, strength, and bone volume (41). This was associated with increased expression of the chondrogenic factor Sox-5 and with increased expression of BMP-2 (41) (Fig. 3A, B). In addition, we recently treated mice with recombinant myostatin propeptide following osteotomy of the fibula, and found that propeptide treatment not only improved soft tissue healing but also increased bone volume in the fracture callus (42-43). The evidence from bone regeneration is therefore consistent with the data from bone marrow-derived stem cells, revealing that loss of myostatin function has direct osteogenic and chondrogenic effects.

Myostatin Inhibitors Are Novel Therapeutic Agents for the Prevention of Bone Fractures

As noted above, myostatin function can be inhibited by follistatin, a myostatin antibody, myostatin propeptide, or a soluble decoy myostatin receptor. Of these myostatin inhibitors the latter three have all been tested in animal models, and the myostatin antibody and soluble receptor have also been studied in human trials. A myostatin antibody (JA16) significantly improved muscle mass and strength in the mdx mouse model of Duchenne muscular dystrophy (44), and a humanized myostatin antibody (MYO-029) was shown to be safe and associated with a moderate trend toward increased muscle size in a phase I/II human trial (45). A neutralizing myostatin antibody, PF-354, was also found to not only increase muscle mass and strength in mice but to also increase treadmill running time and reduce muscle fatigue (46). A recombinant myostatin propeptide attenuated the dystrophic phenotype of mdx mice, and the propeptide stimulated even greater improvements in muscle force than the myostatin antibody (47). These findings are significant from the perspective of fracture prevention for two reasons. First, it is known that children with muscular dystrophy have relatively low bone density, high bone turnover, and are at increased risk for fracture (48-49). Myostatin inhibitors may therefore be particularly effective in treating children with Duchenne muscular dystrophy, as they may not only improve muscle strength but potentially enhance bone mass. Second, while a number of drugs are currently available to treat bone loss, falling is another risk factor for fracture and over 50% of low trauma fractures occur in people who do not have osteoporosis (defined as a T-score ≤ -2.5) (50). There is currently no FDA-approved drug that targets both muscle and bone to decrease frailty and reduce fracture risk. Enhancing muscle mass, strength, and endurance among the elderly is likely to be an effective strategy for improving physical function, reducing the frequency of falls, and decreasing the incidence of fractures.

Recently, several studies have also been reported demonstrating significant anabolic effects of the soluble decoy myostatin receptor (ActRIIB-Fc) on both muscle and bone mass. Lee and colleagues found that the soluble receptor significantly increased muscle mass in mice (16), and a 4-week treatment regime using this same molecule in young mice increased quadriceps mass ~25% and trabecular bone volume in the femur more than 90% (51). Fluorochrome labeling revealed an increase in bone formation rate of more than 300% in the mouse distal femur (51). These findings are impressive, and are consistent with another report in which mice exposed to microgravity on board the Space Shuttle were treated with a decoy myostatin receptor (52). In this study the mice received a single injection prior to the 13 day flight. Untreated mice lost significant bone mass during the flight, whereas mice treated with the decoy receptor and exposed to microgravity did not differ in bone mass from ground control animals. One of the most exciting clinical results is from a randomized placebo-controlled phase I trial involving 48 healthy postmenopausal women who received a single subcutaneous injection of a decoy myostatin receptor. Approximately eight weeks after receiving the injection, women who received the decoy receptor showed increased lean mass, decreased biomarkers of adiposity, increased serum bone-specific alkaline phosphatase, and decreased serum C-terminal type collagen telopeptide (53). These data suggest that the soluble decoy myostatin receptor may improve muscle and bone anabolism in both rodents and humans.

Summary and Conclusions

It is clear that targeting myostatin to prevent fractures and improve bone strength is an intriguing therapeutic strategy, but a number of questions remain. Results from animal studies and human trials in particular suggest that the decoy myostatin receptor is a potent anabolic agent for increasing both muscle and bone mass; however, whereas the myostatin antibody and propeptide are more specific in ligand recognition, the soluble ActRIIB also binds activin, BMP-3, BMP-7, BMP-9, BMP-10, and GDF-11

(28;54;55). It is therefore likely that many of the positive treatment effects observed with the soluble receptor are due to antagonistic effects on other ligands besides myostatin. It is also unclear whether myostatin inhibitors may uncouple bone formation from resorption (for example, by altering OPG production), and while the signaling pathways and downstream factors activated by myostatin in mesenchymal stem cells are becoming better understood, interactions among myostatin, regulatory Smads, and Wnt signaling molecules still are not well-defined. Finally, myostatin is also a pro-fibrogenic factor that can stimulate the expression of TGFβ1 and type I collagen in primary fibroblasts and C2C12 myoblasts (56;57). Congenital absence of myostatin has been associated with weakened tendons in mice (57), and myostatin treatment can enhance tendon regeneration in rats (58). Thus, while myostatin inhibitors may increase muscle mass it is possible that these drugs could also create a "mismatch" between muscle strength and tendon strength, perhaps increasing the risk for tendon tears and ruptures. Long-term studies on the effects of myostatin inhibitors in a variety of musculoskeletal tissues are therefore needed to determine the effects of these molecules on musculoskeletal function. Nevertheless, discoveries to date are promising, and suggest that targeting myostatin (and its receptor) may be an effective therapeutic strategy for improving musculoskeletal function and preventing bone fractures in patients with osteoporosis and neuromuscular disorders.

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