Myostatin as a Potential Therapeutic Target for Obesity and Insulin Resistance

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ABSTRACT

Considering that skeletal muscle is a major tissue responsible for whole body glucose and fat disposal, it has long been speculated that increasing muscle mass would be a potential therapeutic strategy to prevent obesity and fat-induced insulin resistance. Myostatin (growth differentiation factor 8, GDF8) is a transforming growth factor β (TGFβ) family member, and is known to act as a negative regulator of skeletal muscle differentiation and growth. Like other TGFβ members, dimeric myostatin mediates Smad/Mothers against decapentaplegic homolog (SMAD) signal transduction through its specific cell membrane receptors with serine/threonine kinase activity. Myostatin null (Mstn−/−) mice exhibit a doubling of muscle mass due to muscle hypertrophy and hyperplasia, and are protected against fat-induced obesity and insulin resistance. Other genetic and pharmacologic approaches to inhibit myostatin activity also demonstrate an increase in muscle mass and a prevention of obesity and insulin resistance. This review will focus on the effects of myostatin inhibition on obesity and fat-induced insulin resistance, and will discuss the potential underlying mechanisms.

Key words: Myostatin, Muscle growth, Obesity, Insulin resistance

Myostatin:

비만과 인슐린 저항성의 새로운 치료타겟

근육(muscle)은 전신 포도당 및 지방소모에서 결정적으로 가장 중요한 장기인 점을 고려할 때, 근육량(muscle mass)을 증가시키는 것은 비만과 인슐린 저항성을 예방하고 치료하는 효과적인 치료전략으로 오랫동안 고려되어왔다. Myostatin (growth differentiation factor 8)은 transforming growth factor β (TGFβ) 그룹에 속하면서, 최근 근육성장 및 분화를 억제하는 기능을 가진 것으로 알려졌다. TGFβ의 작용기전과 유사하게 myostatin은 세포비임에 존재하는 serine/threonine kinase 활성도를 가진 특이 수용체에 결합하여 SMAD신호전달을 활성화시켜 작용하는 것으로 알려지고 있다. Myostatin 결합 마우스는 근육의 과형성 및 비대를 통해 대조군에 비해 2배의 전신근육 증가를 보였고, 고지방성에 의한 비만 및 인슐린 저항성 발생을 예방하였다. 또한 최근 다른 유전자조작 및 약물로 myostatin 활성을 억제하였을 때도 근육량의 증가 와 함께 비만 및 인슐린 저항성을 억제하는 효과가 보고되었 다. 따라서 본 종설에서는 최근 myostatin활성 억제와 각 작용 기전을 종합적으로 고찰하였다.

중심단어: myostatin, 근육 증가, 비만, 인슐린 저항성

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Background

Obesity and insulin resistance have been known to play an essential role in the development of metabolic syndrome, which prevalence has been dramatically increased, particularly in aged population, over the past decades among advanced and developing societies. Many major factors have been identified and suggested to contribute to the development of obesity and insulin resistance, and among them is a loss of muscle mass known as sarcopenia. Moreover, muscle mass and insulin resistance or components of metabolic syndrome are shown to be closely and inversely related to each other.

Skeletal muscle is a major organ for energy disposal in both humans and rodents, since it represents 40~50% of total body mass and is mainly responsible for insulin-mediated glucose uptake and basal lipid disposal. Muscle glucose uptake is positively correlated with type I muscle fibers and the amount of muscle mass. A decrease in portion of type I muscle fiber has been observed in obesity and type 2 diabetes, and a decrease in muscle volume is also observed in elderly people who are relatively insulin-resistant compared to young people. Therefore, increase in percentage of type I muscle fiber or muscle mass has been widely expected to improve fat-induced obesity and insulin resistance. However, in contrast to the accumulating evidence that increase in type I muscle fiber improves whole body metabolism, relatively little is known about the metabolic effect of increase in muscle mass on obesity and insulin resistance. Despite the lacking evidence, obese and insulin-resistant type 2 diabetic patients are currently encouraged to increase muscle mass with resistance exercise in addition to aerobic exercise.

As a strategy to increase muscle mass, myostatin has been an attractive research subject. Myostatin, a transforming growth factor β (TGFβ) family member protein, has been shown to function as a negative regulator of skeletal muscle differentiation and growth. Myostatin is primarily produced by skeletal muscle cell and acts on muscle tissues in an autocrine manner (Fig. 1). Myostatin homozygous null (Mstn−/−) mice have double...
the skeletal muscle mass compared with normal mice, as a result of a combination of muscle cell hyperplasia and hypertrophy.\textsuperscript{12,13} Due to this ability of controlling the skeletal muscle mass, myostatin might be considered as a good candidate target for the prevention and treatment of obesity and fat-induced insulin resistance.

\textbf{TGFβ Superfamily}

The pleiotropic cytokine TGFβ superfamily of growth factors is composed of related compounds with diverse biologic actions. There are a number of proteins in this family sharing a similar dimeric structure, including TGFβ, growth differentiation factors (GDFs), myostatin (GDF8), bone morphogenetic proteins (BMPs), the activins/inhibins, and Müllerian inhibiting factor (MIF).\textsuperscript{14} The biologic action of the TGFβ superfamily proteins may be altered by non-covalent binding to other proteins. They control various cellular functions including the regulation of cell growth and differentiation as well as cell-cell signaling in diverse cell types of body.\textsuperscript{15}

These actions appear to be exerted through a family of specific membrane receptors with serine/threonine kinase activity.\textsuperscript{16,17} Activin binds to its specific type II receptors, and type II receptor then recruits and forms a heterotetrameric complex with the type IA or IB receptors (also known as activin receptor-like kinases, ALK2 and 4, respectively). There are two activin type II receptors identified: IIA (ActRIIA) and IIB (ActRIIB).\textsuperscript{18,19} Upon ligand binding to the type II receptor, the type I receptor is trans-phosphorylated by the type II receptor, and in turn activates Smad/Mothers against decapentaplegic homolog (SMAD) signal transduction by phosphorylation.\textsuperscript{20} TGFβ and activin receptors phosphorylate C-terminal serine residues of SMADs 2 (Ser465/467) and 3 (Ser423/425)\textsuperscript{21,22}, whereas BMP receptors phosphorylate SMADs 1, 5, and 8\textsuperscript{23,24}, suggesting receptor-specific activation of the SMADs. There are two common-mediator SMADs, SMAD4 and 10, which associate with activated receptor-regulated SMADs (SMAD1, 2, 3, 5, and 8) and translocate into the nucleus.\textsuperscript{25,26} There are also two vertebrate inhibitory SMADs identified. SMAD6 represses the BMP pathway, and SMAD7 inhibits the activin/TGFβ pathway.\textsuperscript{27,29}

\textbf{Myostatin and Myogenesis}

Myostatin is expressed in adult skeletal muscle almost exclusively. Newly synthesized and secreted dimeric myostatin, like many other TGFβ superfamily proteins, becomes the mature form by cleavage of the precursor protein at the tetrapeptide site, and the remaining N-terminal peptide is called propeptide (prodomain), expression of which has an inhibitory effect on myostatin function.\textsuperscript{30} After secretion, myostatin is associated with follistatin that blocks the binding of myostatin to its receptor (Fig. 1). Myostatin eventually binds to the ActRIIs present on myocyte cell surface. It binds to type IIB receptor and, to a lesser extent, type IIA receptor, resulting in a recruitment of ALK4 or 5 to initiate SMAD signaling cascade and an up- or down-regulation of myostatin-specific gene expression associated with myogenesis, such as MyoD and myogenin.\textsuperscript{10,31,32} As a result, myostatin inhibits the differentiation of myoblasts into mature myotubes or muscle fibers. Myostatin also has been shown to inhibit cell proliferation and protein synthesis in C2C12 myoblast cells.\textsuperscript{33}

\textit{Mstn}\textsuperscript{-/-} mice have double the skeletal muscle mass compared with normal mice, as a result of a combination of muscle cell hyperplasia and hypertrophy.\textsuperscript{12} In ActRIIA null (\textit{Acvr2a}\textsuperscript{-/-}) and ActRIIB null (\textit{Acvr2b}\textsuperscript{-/-}) mice, there was an increase in skeletal muscle mass.\textsuperscript{34} Similarly, muscle-specific transgenic expression of follistatin, propeptide, or a dominant negative form of ActRIIB (\textit{Acvr2b}\textsuperscript{DN}) in mice exhibits huge increase in muscle mass.\textsuperscript{35} Application of follistatin\textsuperscript{36} or a monoclonal antibody specific to myostatin\textsuperscript{37} enhances muscle growth, presumably by blocking or neutralizing myostatin, respectively. In addition, functional improvement has been reported in a mouse model of muscular dystrophy by antibody-mediated neutralization of myostatin.\textsuperscript{38} Likewise, administration of soluble ActRIIB leads to a significantly increased muscle mass in mice,\textsuperscript{39} implying that the soluble activin receptor may sequester myostatin and prevent it from interacting with the cell-bound receptors. These studies also support the idea of ActRIIs being cognate myostatin receptors.

Taken together, all these data strongly suggest that myostatin is a key player in inhibiting myogenesis and deficiency of myostatin activity efficiently increases muscle mass. Nevertheless, myostatin does not seem to be
the only muscle growth inhibitor. For example, follistatin is known to antagonize myostatin activity in a latent complex with myostatin (Fig. 1). Transgenic mice with follistatin overexpression (F66) in the background of Mstn−/− have about four times the muscle mass of normal mice, while either F66 or Mstn−/− mice have about twice the muscle mass40, suggesting the existence of at least one other factor that is inhibited by follistatin, which may normally function to limit muscle growth, in addition to myostatin.

However, new potential endogenous factors for the control of muscle development have not yet been identified except myostatin. Moreover, the exact molecular mechanisms for actions of myostatin in regulation of skeletal muscle development have not yet been identified.41 Myostatin a ntagonizes BMP7-induced adipogenesis, although it is unclear whether this effect is stimulatory or inhibitory. Nonetheless, while muscle-specific Acvr2bDN mice show an improvement as described above, adipocyte-specific Acvr2bDN mice demonstrate no effect on body composition, such as lean body mass and fat mass, or weight gain during regular chow and HFD, suggesting an indirect effect of myostatin blockade on adipogenesis in vivo.43)

One likely explanation to support this indirect effect is that an increase in muscle mass by myostatin blockade may lead to an energy shift toward enlarged skeletal muscle from adipose tissue and liver. This shift may allow adipose tissue and liver to support increased energy demand of enlarged skeletal muscle, with increased fat oxidation and glucose utilization, thereby reducing fat accumulation in liver and muscle as well as whole body fat including white adipose tissue. The decrease in fat accumulation may consequently reduce expression of proinflammatory cytokines in adipose and other tissues44, further improving obesity-related pathology.

### Myostatin and Obesity

As suggested above, many reports imply that an increase in muscle mass may be beneficial for obesity. Two lines of Mstn−/− mice show a decrease in fat deposit, fat cell size, liver weight, circulating leptin, triglyceride, and cholesterol levels in both young and increasing age.13,41 A reduction in adipose tissue leptin mRNA, and CCAAT/enhancer binding protein α (C/EBPα) and peroxisome proliferator-activated receptor γ (PPARγ) protein levels in Mstn−/− mice suggest that an increased muscle mass in Mstn−/− mice is associated with reduced adipogenesis.13 Moreover, Mstn−/− mice are protected from high-fat diet (HFD)-induced obesity (DIO)42,43, and Mstn deletion in agouti lethal yellow (Ay) or obese (Lepob-ob) background leads to a partial suppression of fat accumulation.41 In addition, muscle-specific Acvr2bDN mice show increased lean mass and resistance to DIO as well.43)

The effect of myostatin on adipocyte appears to be controversial. Myostatin antagonizes BMP7-induced adipocyte differentiation of 3T3-L1 preadipocyte cell line and blocks adipogenesis44, by reduction in glycerol-3-phosphate dehydrogenase (GPDH) activity, and C/EBPα and PPARγ protein levels, and induction in SMAD2 phosphorylation (Ser465/467), thereby inhibiting adipogenesis.5,46 On the other hand, myostatin treatment is not able to alter lipolysis in fully differentiated 3T3-L146, and other studies report an induction of adipogenesis by myostatin.47 These in vitro studies imply that there seems to be a direct function of myostatin on adipocyte during adipogenesis, although it is unclear whether this effect is stimulatory or inhibitory.
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(TNFα) production and a reduction in proinflammatory cytokine gene expressions, such as Tnfα, Il1β, F4/80, and Gpr43 in adipose tissue and skeletal muscle, and CD36 in liver. Aged Mstn1+/−2+ mice are also protected against overall insulin resistance in liver and peripheral tissues during the hyperinsulinemic-euglycemic clamp studies at 11-month-old on an HFD.48)

Another approach for disruption of myostatin function is using myostatin propeptide. Unlike Mstn−/− model, however, transgenic mice with muscle-specific expression of the myostatin propeptide increase muscle mass with no difference in white adipose tissue mass, while showing an improvement of insulin sensitivity during DIO.49)

There also have been several pharmacologic approaches to understand the relationship between muscle mass and insulin sensitivity, such as neutralizing antibodies37,38,50) and decoy receptors to sequester myostatin34,39,51), with more profound effect in the latter. Myostatin putatively binds to the ActRIIB, and a soluble form of long-lasting ActRIIB generated by fusion between extracellular domain of the receptor and Fc region of immunoglobulin G (ActRIIB/Fc, RAP-031) has been used as a decoy receptor of myostatin.34) Injection of ActRIIB/Fc for 4 weeks causes an increase in muscle mass in regular chow-fed mice34) as well as in HFD-fed mice.39) ActRIIB/Fc injection also enhances insulin sensitivity (increase in glucose infusion rate and decrease in hepatic glucose production) under clamp conditions in HFD, and ActRIIB/Fc injection for 10 weeks further increases muscle mass and drastically reduces fat content in a HFD setting.

All of the above studies strongly support the idea that increasing muscle mass by genetic or pharmacologic blockade of myostatin may prove to be beneficial for prevention and treatment of obesity and insulin resistance. Several possibilities should be considered as mechanisms by which deficiency of myostatin activity increases insulin sensitivity and prevents fat-induced insulin resistance. First, many studies suggest that lipid accumulation in muscle and liver, two major organs for glucose metabolism, causes insulin resistance in these tissues.52-54) Thus, increase in muscle mass by deficiency of myostatin activity may enhance energy metabolism, resulting in decreased fat accumulation in muscle and liver, which may, in turn, prevent fat-induced insulin resistance as seen in several studies.13,41,43) Second, Mstn−/− and F66/Mstn−/− mice, which have doubled and quadrupled muscle mass respectively, show markedly increased peripheral glucose uptake43,55), suggesting that enlarged muscle mass per se may be the main reason for increased peripheral glucose uptake. Another possibility is that altered adipokines or proinflammatory cytokines due to decreased adipose tissue and/or reduced fat accumulation in skeletal muscle and liver with loss of myostatin function48,49) may change insulin response in these tissues.

Myostatin and Hepatic Glucose Metabolism

Many studies mentioned above suggest that an improved hepatic insulin resistance may be a secondary change to increased energy metabolism in enlarged skeletal muscle mass and/or altered cytokines from adipose tissue. However, there is some evidence suggesting that myostatin is also able to directly activate SMAD signaling in hepatocyte cell line. In a human hepatic carcinoma cell line, HepG2, myostatin increases SMAD2 phosphorylation and enhances transactivation of a promoter containing SMAD-binding element.19) HepG2 cells express ActRIIA, ActRIIB, and ALK4 receptors, and it appears that myostatin mediates its signaling through these receptors.

Thus, it can be speculated that inhibition of myostatin may result in a reduction of hepatic SMAD signaling, and as a consequence, hepatic insulin sensitivity may be enhanced. However, despite of the possibilities of a direct effect of myostatin on hepatocytes, it is yet unclear whether reduced SMAD activation and enhanced insulin action in hepatocytes are connected to each other to influence hepatic glucose metabolism. The cellular and molecular mechanisms underlying myostatin action in hepatic glucose metabolism are yet to be investigated.

Conclusion and Significance

Natural mutations in myostatin gene have been reported in humans56) as well as domesticated animals.57-60) All of these mutations have shown to cause a muscular hypertrophy, implying a strong feasibility to develop myostatin-related substances as drugs for the treatment of muscular dystrophy. More importantly, as described above, several recent reports demonstrating a beneficial effect of myostatin loss-of-function on obesity and insulin
resistance suggest that myostatin may have a great potential as a novel target for treatment of obesity and type 2 diabetes.

In conclusion, these studies will significantly contribute to and serve as valuable information for therapeutic strategies in the development of novel multi-purpose drugs and in the treatment of muscle diseases, obesity, diabetes, and other metabolic disorders.

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