

ABSTRACT OF DISSERTATION

Title	Effects of Co-Transfection with Myostatin-Targeting siRNA and ActRIIB-Fc Fusion Protein on Skeletal Muscle Growth (Myostatin-siRNA および ActivinIIB 型受容体融合タンパク質の共導入による骨格筋形成制御効果の検討)
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<p>Background:</p> <p>Recent advances in the field of muscle biology have led to new interest in the pharmacological treatment of muscle wasting. Myostatin (Mstn) is a member of the transforming growth factor-β (TGF-β) superfamily of growth and differentiation factors. The expression pattern of Mstn suggests that it plays a role in regulating muscle development and function. RNAi is a high sequence-specific gene silencing technique, in which short pieces of double-stranded RNA, small interfering RNA (siRNA), suppress the expression of the genes exhibiting sequence homology. We identified a siRNA region that targeted Mstn for degradation. On the other hand, Activin type IIB receptor (ActRIIB) is a type II TGF-β superfamily receptor known as a key player in the regulation of muscle size and strength. Mstn binds to the ActRIIB, which regulates the Smad signaling pathway to inhibit MyoD and myogenin expression and to decrease the movement of myogenic stem cells from G to S phase. The soluble ligand-binding domain of ActRIIB fused to the Fc domain of IgG (ActRIIB-Fc) potently binds and inhibits TGF-β family members in muscle, leading to rapid and marked muscle growth. The present study was designed to assess the combinative effects of myostatin-targeting siRNA (Mstn-siRNA) and ActRIIB-Fc on murine myoblast <i>in vitro</i> and <i>in vivo</i>.</p> <p>Materials and Methods:</p> <p>C2C12 cells were treated by Mstn-siRNA with or without ActRIIB-Fc at 0 and 48 h after differentiation. Myotube size was measured, and gene expression of <i>Mstn</i>, <i>MuRF-1</i>, <i>MyoD</i> and <i>myogenin</i> were analyzed. Furthermore, 11-week-old, male C57BL/6 mice were injected with atelocollagen (ATCOL)-mediated Mstn-siRNA and Mstn-siRNA/ActRIIB-Fc locally into the masseter muscle twice a week. Histological and biochemical analyses were performed using the dissected muscle.</p> <p>Results:</p> <p>Transfection of Mstn-siRNA and Mstn-siRNA/ActRIIB-Fc resulted in significant</p>	

increases in the myotube diameter of the C2C12 cells compared with untreated control. Also, treatment with Mstn-siRNA and Mstn-siRNA/ActRIIB-Fc could lead to an upregulation of *MyoD* and *myogenin* gene expression and downregulation of *Mstn* and *MuRF-1*. *In vivo*, muscle fibril hypertrophy was observed in both Mstn-siRNA and Mstn-siRNA/ActRIIB-Fc treated groups. Moreover, western blotting analysis showed that the p-Smad2/3 expression level was decreased by treatment of Mstn-siRNA/ActRIIB-Fc. In contrast, MyoD and myogenin protein levels were increased by combined treatment, compared with the other groups.

Discussion and Conclusions:

Our study indicated that co-Mstn-siRNA/ActRIIB-Fc treatment increased the murine myotube size through an upregulation of regulatory genes responsible for myogenesis, such as *MyoD* and *myogenin* leading to myoblast fusion and myotube maturation. We also found that the *Mstn* and *MuRF-1* (atrophy-related gene) mRNA expression levels were equally downregulated by Mstn-siRNA alone and Mstn-siRNA plus ActRIIB-Fc combination. Previous study reported that *Mstn* upregulates atrophy-related gene through FOXO, leading to muscle atrophy. In addition, Mstn inhibits myogenic differentiation by downregulating *MyoD* and *myogenin* expression.

In our *in vivo* study, results also showed that the increased muscle fibril size was due to the inhibition of Mstn signal by the combined administration. The two components of this combination with different mechanisms of suppression of Mstn signaling seem to produce synergistic effects. The p-Smad2/3 protein expression was equally downregulated by Mstn-siRNA alone and Mstn-siRNA plus ActRIIB-Fc combination. These results suggest that ActRIIB-Fc does not affect the expression of Mstn in the masseter muscle and that the mechanism of action of the combination of Mstn-siRNA and ActRIIB-Fc is different from that of ActRIIB-Fc. Moreover, MyoD and myogenin protein levels were increased in masseter muscles with Mstn-siRNA and ActRIIB-Fc administration compared with the untreated control and individual treatment. The double inhibition of Mstn with different suppression mechanisms seems to produce synergistic effects. These suggest that double inhibition of myostatin is potentially useful for myogenesis and muscle growth promotion.

This may be a good as new treatment remedy for patients with various muscle atrophies, including muscular dystrophy.

Key words: myostatin, small-interfering RNAs, activin type IIB receptor, muscle hypertrophy