Expression of Activin βA and Mighty during Skeletal Muscle Atrophy

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ABSTRACT

Myostatin, a TGFβ superfamily member, is an important modulator of myogenesis. While deletion of myostatin gene results in double-muscling phenotype, over-expression of myostatin gives rise to muscle wasting. Another negative regulator of myogenesis alongside myostatin is Activin, both being the TGFβ superfamily member, share the same signaling pathway via ligand-binding to Activin type IIB receptor (ActRIIB). Activin has been identified to regulate cell proliferation and differentiation. However, its precise role in postnatal skeletal muscle and atrophy is not known. Therefore, one of the aims of this study is to analyze the gene expression of Activin βA subunit and its receptor, ActRIIB in postnatal muscle at different stages of myogenesis and during atrophy. The results showed an elevated expression of Activin βA in myoblasts compared to myotubes. Upregulation of Activin βA levels observed in myostatin-null hypertrophy model also signifies that Activin βA plays a role in regulating muscle growth in the absence of myostatin. Thus to fully investigate the role of Activin βA, full-length Activin βA was also cloned from murine muscle for producing recombinant protein and for studying its expression in mammalian cells. In addition, the expression of a novel promyogenic factor, Mighty, in atrophy model was also analyzed.

INTRODUCTION

Myogenesis, the formation of skeletal muscle tissues, is negatively regulated by Myostatin, a member of the TGFβ superfamily. Deletion of Myostatin gene results in a double-muscling phenotype whereas overexpression of Myostatin leads to muscle wasting (Kambadur et al, 1997). Myostatin signals via receptor ActRIIB. Another TGFβ superfamily member, Activin βA also

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signals via receptor ActRIIB regulating its downstream target genes. It is postulated that Activin βA is another regulator of myogenesis which acts in conjunction with Myostatin in preventing excessive skeletal muscle growth (He et al, 2005). Hence, its precise role in regulating skeletal muscle development and muscle wasting needs to be explored.

Mighty, a downstream target gene of Myostatin, functions as a promyogenic factor in skeletal myogenesis (Marshall et al, 2008). The gene expression of this novel gene is hypothesized to be negatively regulated by Myostatin levels, predominant in skeletal muscles (Marshall et al, 2008). Thus, its gene expression with correlation to Myostatin levels needs to be determined.

Therefore, the aim of this present study is to analyse the gene expression of Activin βA, ActRIIB and Mighty in the different stages of postnatal mammalian muscle development and in conditions of skeletal muscle wasting. Full-length Activin βA and Mighty ORFs were also cloned from murine muscle samples for future production of overexpressing Activin βA and Mighty cell lines respectively. These recombinant genes will be important to further elucidate the respective functions of Activin βA and Mighty gene in myogenesis.

MATERIALS AND METHODS

Total RNA was isolated from muscle samples using TRIzol reagent (Invitrogen) and quantified using NanoDrop™ 1000 Spectrophotometer. Reverse transcription was conducted using SuperScript® First Strand Synthesis System (Invitrogen). Murine and human Activin βA, murine ActRIIB, murine Mighty, murine α-tubulin were amplified by Polymerase Chain Reaction (PCR). Restriction enzyme digestion was set up with HindIII, NcoI and XcmI for Activin βA, ActRIIB and Mighty respectively. Full-length Activin βA ORF was amplified from wild-type murine primary myoblasts, characterized with HindIII, cloned and sequenced.

RESULTS

Activin βA fragment was amplified from 5-day-old and 15-year-old human myoblasts. Activin βA was further characterized by restriction digestion with HindIII. Gene expression analysis was performed on myostatin-null hypertrophy model as well as myostatin-induced and dexamethasone-induced atrophy models at postnatal stage. Full-length Activin βA ORF was amplified from wild-type murine primary myoblasts, characterized with HindIII, cloned and sequenced.
Mighty fragment was amplified from murine primary myoblasts and characterized with XcmI. Gene expression of Mighty was analysed on dexamethasone-induced atrophy model. Full-length Mighty ORF was amplified from murine gastrocnemius muscle and cloned.

**DISCUSSION**

In this present study, Activin βA gene has been amplified from murine and human myoblasts thus confirming its presence in mammalian postnatal skeletal muscles. Gene expression of Activin
βA and its receptor ActRIIB were also studied on myostatin-null hypertrophy model and muscle wasting model. The differential expression of Activin βA and ActRIIB were observed to be higher in the myostatin-null than wild-type TA muscle. Thus, it is speculated that Activin βA might have undergone a compensatory mechanism to upregulate its own expression in absence of Myostatin. On the other hand, sensitivity of ActRIIB receptor increased upon ligand-binding with Activin βA.

Expression of Activin βA was significantly higher in 24-hours proliferating C2C12 myoblasts as compared to 96-hours differentiating C2C12 myoblasts. This suggests Activin βA confers an inhibitory role in skeletal muscle development in repressing differentiation of myoblasts into myotubes (Link and Nishi, 1997). In the myostatin-induced atrophy model, the differential expression for both Activin βA and ActRIIB were insignificant. Thus, their expressions at protein levels need to be determined. In the dexamethasone-induced atrophy model, gene expressions for Activin βA, ActRIIB and Mighty were observed to be downregulated in a dose-dependent manner (Ma et al, 2003). It is postulated that Activin βA might confer an auto-regulatory feedback loop, similar to Myostatin. On the other hand, it is postulated that Mighty expression might have been downregulated by high Myostatin levels associated to glucocorticoid administration, thus implicates its role as downstream target gene of Myostatin (Marshall et al, 2008).

Overall, it is postulated that Activin βA might play a compensatory role in maintaining muscle size in absence of Myostatin since both function as negative regulators of myogenesis. On the other hand, the novel promyogenic gene, Mighty, is speculated to confer a profound role in offering resistance to muscle wasting.

REFERENCES