Inhibition of Myostatin: A New Approach to Improve Skeletal Muscle Healing after Injury

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Introduction

Skeletal muscle injuries are the most common injuries encountered in sports medicine. Muscle injuries can heal spontaneously through regeneration, but fibrosis impedes this process and results in incomplete functional recovery. The recent identification of myostatin (MSTN), a member of the TGF-β superfamily[1], may facilitate the development of new therapies by which to promote muscle healing. MSTN is a potent negative regulator of muscle growth. MSTN expression occurs predominantly in the skeletal muscle. In this study, we first examined whether the absence of MSTN reduces fibrosis in injured skeletal muscle of MSTN knockout (MSTN-/-) mice; second, we investigated whether an adeno-associated virus 2 (AAV2) vector delivered MSTN propeptide (MPRO), a natural antagonist of MSTN, can block MSTN and improve the healing of injured muscles of wild-type (WT) mice by enhancing regeneration and reducing fibrosis.

Materials and Methods

All experiments in this study were in accordance with research protocols approved by the ARCC of Children's Hospital of Pittsburgh.

Muscle healing in MSTN-deficient muscles: MSTN-/- mice and C57BL/6 WT mice (controls) underwent bilateral gastrocnemius muscle (GM) laceration (n=6 per group). GMs of both MSTN-/- and WT mice were harvested 4 weeks after laceration.

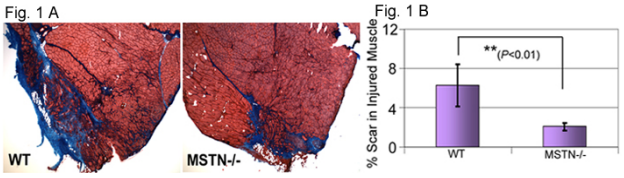
Muscle healing in the skeletal muscle transduced with AAV2-MPRO: C57BL/6 WT mice were used for these experiments (n=5). AAV2-MPRO ($1x10^{11}$ v.g.) was injected into the left GM of each mouse; the same amount of phosphate buffered saline (PBS) was injected into the contralateral GM as a control. Ten days after AAV2 vector transduction, both GMs of each mouse were lacerated. The GMs were harvested 4 weeks after laceration.

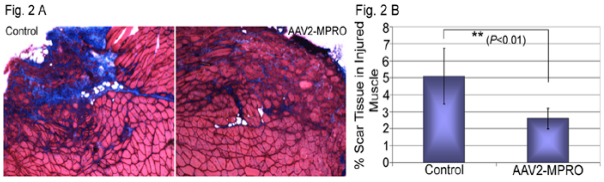
Data analysis: Masson's Trichrome staining (nuclei [black], muscle [red], collagen [blue]) was performed to identify fibrotic scar tissue in the injured muscles. Northern Eclipse software was used to measure areas of fibrotic tissue and diameters of regenerating muscle fibers within the scar tissue. Student's t-test was used to determine significance (P<0.05).

Results and Discussion

The injured skeletal muscle of the MSTN-/- mice contained significantly less fibrous scar tissue than the injured muscle of the WT mice (Fig. 1 A, B). The regenerating myofibers were significantly larger in diameter than those in the injured muscle of WT mice (data not shown). These findings suggest that muscle regeneration is enhanced in MSTN-/- mice compared with WT mice.

Our results are consistent with previous findings showing that *mdx* mice (which model Duchenne muscular dystrophy) subjected to MSTN gene knockout (MSTN-/-/mdx) show both better regeneration and less fibrosis than normal *mdx* mice (MSNT+/+/mdx) [2]. McCroskery et al. recently reported reduced fibrosis in the notexin-damaged tibialis anterior muscles of myostatin-null mice compared with similarly damaged muscles in WT mice [3]. Our previous research provides additional evidence that MSTN stimulates fibrosis. We have demonstrated that MSTN stimulates proliferation and myofibrotic differentiation of fibroblasts (unpublished data), which is responsible for the excessive accumulation of extracellular matrix in the injured muscle. These results strongly suggest that MSTN plays an important role in fibrosis after skeletal muscle injury and, therefore, that MSTN, like TGF-β1, should be a target of antifibrosis therapies.





The study reported here also shows the use of AAV2-mediated gene transfer to introduce MPRO into GMs in vivo. Figure 2 shows significantly less fibrous scar tissue in the AAV2-transduced GMs than in the contralateral GMs. Moreover, the diameters of regenerating myofibers in GMs treated with MPRO tended to be larger than those in nontreated GMs, although the difference was not significant (data not shown). The improved muscle healing resulting from the blockade of MSTN is comparable to that seen in MSTN-/- mice.

Conclusions

Our results indicate that MSTN stimulates fibrosis in injured muscle and that the direct delivery of AAV2-MPRO into skeletal muscle is an effective approach by which to improve muscle healing.

References

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