FOLLISTATIN IMPROVES SKELETAL MUSCLE HEALING AFTER INJURY

INTRODUCTION

Skeletal muscle injuries account for 10 to 30% of all sports related injuries. Muscle injuries can heal via spontaneous regeneration; however, as a result of severe injury, incomplete functional recovery can increase the time an athlete is off the field. Decorin has been shown to effectively improve skeletal muscle healing by reducing fibrosis and accelerating skeletal muscle regeneration [1]. Aside from decorin, Follistatin (FLST) has been drawing more attention to the fact that increasing knowledge about this molecule reveals it as a promising new target for therapeutic improvement of skeletal muscle healing. FLST was initially described as an inhibitor of follicle-stimulating hormone decades ago. More and more evidence revealed that FLST is capable of binding and neutralizing many members of the TGF-β superfamily such as myostatin (MSTN) and activin [2, 3]. Recently, it was found that FLST overexpression mice (FLST OE) showed dramatic increase in skeletal muscle mass compared to wild-type control mice [4]. Activin is implicated in the formation of fibrosis in many tissues and organs such as skin, liver, and kidney [2, 3]. Furthermore, our unpublished data demonstrated that MSTN is a fibrosis stimulator in the skeletal muscle and blocking MSTN significantly reduces the formation of fibrosis in injured skeletal muscle of mice. Taken together, we hypothesize that FLST may improve the healing of injured skeletal muscle. In this study, transgenic FLST OE mice were used to investigate the influence of FLST on skeletal muscle healing.

METHODS

Myoblast differentiation assay: C2C12 myoblasts were plated onto collagen-coated 12-well plates with normal medium overnight. The following day, the medium was replaced with low serum medium (2% horse serum) plus different concentrations of FLST (Sigma, St. Louis, MO). The cells were cultured for 6 additional days. Medium and recombinant FLST protein were changed every other day. Myotubes were monitored by myosin heavy chain (MHC) immunostaining, and the fusion capacity of C2C12 myoblasts was evaluated by determining the number of myonuclei per myotube (n=3).

Western blot: C2C12 myoblasts were plated onto collagen-coated 6-well plates. Following overnight attachment, normal medium was replaced with low serum medium in the presence of decorin (Sigma, St. Louis, MO). Protein samples were collected at 48 and 72h and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Goat anti-follistatin antibody (Santa Cruz Biotechnology, INC: Santa Cruz, CA) was used to detect FLST expression in C2C12 myoblasts.

Animal model: All experiments in this study were approved by the Children’s Hospital of Pittsburgh IACUC. C57BL/6j wide-type (WT) and FLST OE mice (7 to 8 weeks of age) were used for all experiments (n=6). Both gastrocnemius muscles (GMs) of each mouse underwent bilateral laceration [1]. The GMs were harvested 4 weeks after GM laceration. This suggests that FLST is involved with an alternative mechanism for skeletal muscle healing. Moreover, we also showed that decorin treatment elevated FLST expression in C2C12 myoblasts. It seems likely that, apart from neutralizing TGF-β1, decorin also reduces fibrosis in injured skeletal muscle by up-regulating the intermediate molecule, FLST. The skeletal muscle healing process is a complex process, in which many molecules interact with each other, and our findings provide insight into one aspect of this network.

RESULTS

FLST induces large myotube formation in vitro: Our results reveal the ability of FLST to stimulate myogenic differentiation as shown in Fig 1. Myotubes treated with FLST were larger and contained more nuclei than the control (Fig 1A). FLST treatment leads to a significant increase in the number of nuclei per myotubes in a dose dependent manner (Fig. 1B) compared to control, suggesting that FLST promotes myogenic fusion and accelerates the maturation of myotubes.

Reduced fibrosis in FLST OE mice after GM laceration: The results of Masson’s Trichrome histochemistry revealed significantly less fibrous scar tissue in the FLST OE GMs than in the WT GMs (Fig 2A, B).

DISCUSSION

We have demonstrated that TGF-β1 plays a significant role in both the initiation of fibrosis and the induction of myofibroblastic differentiation by myogenic cells in injured skeletal muscle [6]. Although, FLST improves skeletal muscle healing, it does not seem to block fibrosis in injured skeletal muscle through inhibition of TGF-β1. Our result showed that FLST failed to block the inhibition that TGF-β1 has on myogenic differentiation (data not shown). Most likely, FLST inhibits the formation of fibrosis by antagonizing the activities of activin and MSTN [2, 3]. FLST overexpression improves skeletal muscle healing by reducing fibrosis. Although FLST significantly increases myoblast differentiation in vitro, FLST OE mice did not show increased diameter of regenerating myofibers compared to WT mice at 4 weeks after GM laceration. This suggests that FLST is involved with an alternative mechanism for skeletal muscle healing. Moreover, we also showed that decorin treatment elevated FLST expression in C2C12 myoblasts. It seems likely that, apart from neutralizing TGF-β1, decorin also reduces fibrosis in injured skeletal muscle by up-regulating the intermediate molecule, FLST. The skeletal muscle healing process is a complex process, in which many molecules interact with each other, and our findings provide insight into one aspect of this network.

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REFERENCES


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