DECORIN PROMOTES MUSCLE REGENERATION

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Introduction:
Decorin is a small chondroitin/dermatan sulfate proteoglycan composed of a ~38 kDa core protein found as a constituent of the extracellular matrix of all collagen-containing tissues[^1]. Other studies have shown that decorin binds to transforming growth factor β1 (TGF-β1), a growth factor which is responsible for initiating fibrosis in many tissues[^2]. In our laboratory, we have observed that the direct injection of human recombinant decorin at 2 weeks after muscle laceration has resulted in an effective prevention of muscle fibrosis and near-complete functional muscle recovery[^3]. The ability of decorin to inhibit TGF-β, is a likely mechanism by which decorin prevents scar tissue formation[^4,5]. In this study, we hypothesize that decorin has, in addition to an ability to inhibit the fibrosis of skeletal muscle, an ability to enhance skeletal muscle regeneration. In order to test this hypothesis, we first transfected myoblasts (C2C12) with a decorin plasmid. *In vitro*, we then compared the rate of differentiation of these cells into myotubes with the rate at which normal myoblasts differentiate and form myotubes. *In vivo*, we have attempted to make a similar comparison between the effect of myoblasts transfected with decorin versus the effect of normal myoblasts on the ability of the injected cells to regenerate skeletal muscle.

Methods:
1. In Vitro:
An AAV-mDec plasmid, spliced with mouse decorin sequence, which was enhanced by a CMV promoter, was transfected into C2C12 cells by lipofectin. The C2C12 transfected cells (CD) were cultured for two weeks in DMEM containing 500 µg/ml of G418 and 10% horse serum (Gibco BRL). CD cells were cultured in DMEM with 500 µg/ml of G418. For fusion, both C2C12 and CD cells were cultured in DMEM free of serum. The number of total myotubes and the number of large myotubes (greater than three nuclei) was measured in both CD and C2C12 wells by sampling numerous microscopic fields. All of the cells were grown at 37°C in 5% CO2.

2. In Vivo:
C2C12 and CD clone cells were transfected with a LacZ retrovirus vector. LacZ positive CD (1x10⁶) cells were injected into the gastrocnemius muscles (GMs) of SCID mice in the left legs. The GMs of the right legs were injected with the same number of C2C12 cells (LacZ positive) as a control. The GMs were analyzed by histology and immunohistochemistry from 1 to 6 weeks post-transplantation.

Results:
1. Decorin increased myoblast differentiation *in vitro*.
We observed that CD cells grow and proliferate at a similar rate as C2C12 cells, however CD clone cells differentiate and rapidly fuse into larger myotubes at a faster rate than C2C12 cells when cultivated in differentiation medium (Fig. 1). CD cells also demonstrate a higher expression of myosin than C2C12 cells upon differentiation *in vitro* (Fig. 2).

2. Decorin enhanced regeneration in skeletal muscle after myoblast transplantation.
Both CD and C2C12 cells gradually fused into local myofibers post-injection. However, CD cells fuse with more myofibers when compared to C2C12. Transplantation of C2C12 cells results in smaller myofibers with more connective tissue (Fig. 3). Morphometric analysis shows an increased level of cell differentiation *in vitro* and regeneration *in vivo* is induced by decorin. The increased incorporation of CD cells (donor myoblasts) in the injected site may be due, at least in part, to an increased level of regeneration.

Discussion:
Previous findings have already shown that decorin has a potential to improve muscle healing to a near-complete functional recovery after muscle injury, however our results were attributed to the anti-fibrosis effect of decorin[^3]. In this study, our results show that decorin can also act as a potent stimulator of skeletal muscle regeneration. Myoblasts expressing decorin are able to differentiate and fuse into myofibers at a significantly higher rate than normal myoblasts, both *in vitro* and *in vivo*.

Myoblasts and muscle satellite cells expressing decorin at an injured site should be capable of regenerating damaged myofibers in a more effective manner as compared to regular myoblasts. Other studies have shown that decorin interacts with the cell regulator gene p21[^6], which is a possible explanation for the ability of decorin to arrest fibroblast growth[^7]. This study shows that decorin also has a significant effect on myoblasts. *In vitro*, decorin transfection caused myoblasts to form a greater number of myotubes which were also much larger than normal. *In vivo*, myoblasts transfected with decorin promoted muscle regeneration. These results suggest that the improvement of muscle healing following decorin injection is not only attributed to its anti-fibrosis action but also to its ability to enhance muscle regeneration.

References:

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