THE MECHANISM BEHIND DECORIN'S EFFECT ON MYOBLAST DIFFERENTIATION: INTERACTION BETWEEN DECORIN AND THE MYOGENIC GENES MYOSTATIN AND P21

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INTRODUCTION

Previously, we have demonstrated that decorin can improve injured skeletal muscle healing by both blocking the fibrotic effect of transforming growth factor beta (TGF-beta) and enhancing muscle regeneration (1,2). We now are working to identify the underlying mechanisms by which decorin enhances muscle regeneration. We have determined that decorin increases muscle cell fusion and up-regulates myogenic gene expression during myoblast differentiation. However, the mechanism by which decorin stimulates the signaling pathway responsible for this up-regulated differentiation remains unknown. In the study reported here, we genetically modified myoblasts to express the decorin gene to investigate the mechanisms behind decorin's effect on muscle regeneration.

MATERIALS AND METHODS

Cell culture: C2C12 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and 10% horse serum (HS) (Gibco BRL). To promote differentiation, the cells then were cultured for up to 6 days in DMEM supplemented with 2% HS, at which point the myotubes were counted. All cells were grown at 37^0C in 5% CO_2.

Gene transfer: Lipofectamine was used to transfect C2C12 cell with an AAV-mDecorin plasmid containing a mouse decorin sequence and a CMV promoter. The transfected C2C12 cells, 'CD cells', were selected by treatment with G418 (500 mg/ml) for 2 weeks. The expression of decorin by the CD cells was verified by western blot analysis.

Western blot analysis: C2C12 and CD cells were lysed when the cultures reached 70% confluence. Protein samples were separated on a 12% sodium dodecyl sulfate-polyacrylamide electrophoresis gel, transferred to nitrocellulose membranes, and analyzed by immunostaining. The primary antibodies used for this process were anti-decorin (gift from Dr. Fisher, NIH) at 1:1000; anti-myf5, anti-myf6, anti-myoD, anti-myogenin, and anti-myostatin (Santa Cruz Bio., Inc.) at 1:1000; and anti-myosin, anti-desmin, and anti-P21 (Sigma) at 1:2000 for 1 hour at room temperature. The secondary antibody, anti-rabbit-HRP (1:5000, Santa Cruz Bio., Inc.), was applied for 1 hour. Peroxidase activity was determined by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech), and positive bands were detected on X-ray film.

RESULTS

1. Decorin-transfected C2C12 cells expressed high levels of decorin (Fig. 1). After selection with G418, CD cells expressed high levels of decorin protein, as demonstrated by western blot analysis.

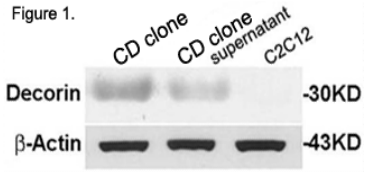

Figure 1.

2. Decorin increased myoblast differentiation by increasing the expression of myogenic proteins in vitro (Fig. 2A). In comparison with nontreated C2C12 cells, CD cells exhibited greatly enhanced differentiation and fusion. Western blot analysis revealed increased expression of the myogenic proteins myf5, myf6, myoD, and myogenin in the CD cell population. However, desmin expression by the 2 cell populations remained similar.

3. Decorin up-regulated P21 but down-regulated myostatin expression in myoblasts (Figs. 2B and 2C). We detected a low level of P21 expression in the C2C12 cells; however, we observed a high level of P21 expression in the CD cells (**Fig. 2B**). In addition, C2C12 cells expressed a detectable level of myostatin whereas CD cells did not (**Fig. 2C**).

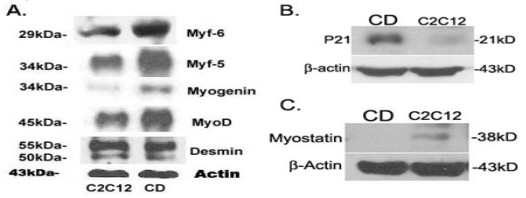

Figure 2.

DISCUSSION

Decorin, a small leucine-rich proteoglycan, is a key regulator in extracellular matrix assembly and cell proliferation (3,4). Decorin also has a specific effect on the migration and differentiation of embryonic skeletal muscle cells (5). We have demonstrated that decorin can prevent muscle fibrosis, enhance muscle regeneration, and improve the functional recovery of injured skeletal muscle (1,2). We also have determined that decorin enhances muscle regeneration by establishing or coordinating muscle growth after muscle injury. The results reported here demonstrate that decorin can up-regulate P21, an important cyclin-dependent kinase (cdk) inhibitor, but down-regulates myostatin, a negative regulator of skeletal muscle mass, in myoblasts (6,7) (**Fig. 3**). The up-regulation both of P21 and of other myogenic genes, including MyoD, in decorin-expressing muscle cells is a potential mechanism by which decorin promotes muscle cell differentiation and muscle regeneration; indeed, research has shown that myoD plays a central role in the differentiation of muscle cells (8). Moreover, the down-regulation of myostatin also might help to accelerate skeletal muscle healing after injury. In sum, our results indicate that decorin influences muscle healing by up-regulating the expression of both P21 and myogenic proteins, including MyoD, while down-regulating the expression of TGF-beta1 and myostatin (**Fig. 3**).

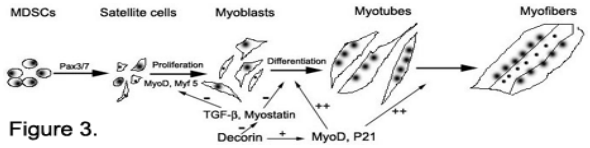

Figure 3.

ACKNOWLEDGMENTS

The authors wish to thank Marcelle Pellerin and James Cummins for technical assistance. This work was supported by funding from the Henry J. Mankin Endowed Chair for Orthopaedic Research at the University of Pittsburgh, the William F. and Jean W. Donaldson Chair at Children's Hospital of Pittsburgh, the Hirtzel Foundation, and a grant awarded to Dr. Johnny Huard from the National Institutes of Health (R01 AR47973).

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