Available online at www.sciencedirect.com
SCIENCE DIRECT
Comparative Biochemistry and Physiology, Part A 144 (2006) 501–508
CBP
www.elsevier.com/locate/cbpa

Effect of myostatin on turkey myogenic satellite cells and embryonic myoblasts

Douglas C. McFarland [a,*], Sandra G. Velleman [b], Jane E. Pesall [a], Caini Liu [b]

[a] Department of Animal and Range Sciences, Box 2170, ASC 101, South Dakota State University, Brookings, SD 57007-0392, USA
[b] Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691, USA

Received 12 January 2006; received in revised form 14 April 2006; accepted 19 April 2006
Available online 30 April 2006

Abstract

Myostatin (GDF-8) inhibits the activation, proliferation, and differentiation of myogenic satellite cells. The relative importance of this growth factor is demonstrated in myostatin-null mice and cattle possessing defective myostatin genes. These defects result in greatly enhanced musculature. In the present study, we examined the effect of myostatin on turkey myogenic satellite cells and embryonic myoblasts. Compared with controls ($P<0.05$), proliferation of both turkey embryonic myoblasts and satellite cells was inhibited between 26 and 45% in serum-free medium containing 20 ng/mL myostatin. While individual turkey satellite cell clones differed in their responsiveness to myostatin, there were no significant differences in the responsiveness of fast and slow growing cells as groups ($P>0.05$). A slow growing clone that exhibited the greatest response to myostatin also exhibited the greatest depression of differentiation with this growth factor ($P<0.05$). All other turkey satellite cell clones exhibited similar responses to the differentiation depressing effects of myostatin ($P>0.05$). However, myostatin had no effect on differentiation of turkey embryonic myoblasts ($P>0.05$). When exposed to myostatin, 4 of 6 proliferating clones and all differentiating clones increased their expression of decorin, a growth inhibitor ($P<0.05$). The present study demonstrates that myostatin inhibits the proliferation and differentiation of satellite cells and suggests a role for decorin in myostatin action in muscle development.

Keywords: Muscle; Myostatin; Decorin; Turkey; Satellite cell; Myoblast; Proliferation; Differentiation; Gene expression

1. Introduction

Early embryonic development of skeletal muscle results from the proliferation, differentiation, and fusion of embryonic myoblasts within newly forming muscle beds. Postnatal/posthatch muscle development is largely the result of the activity of myogenic satellite cells associated with muscle fibers. Residing between the basement membrane and plasmalemma of muscle fibers (Mauro, 1961; Muir, 1970), satellite cells proliferate, differentiate, and fuse with the adjacent fibers (Moss and LeBlond, 1971). The major substances regulating this process are growth factors (for review: McFarland, 1999). Another important factor impacting the activity of satellite cells and the development of skeletal muscle is the extracellular matrix (ECM). In addition to serving as a structural scaffold for tissue and organ growth, the ECM appears to also regulate cell behavior by interacting with growth factors and through cellular signal transduction pathways (for review: Velleman, 1999).

Expression of decorin, a proteoglycan component of the ECM, has been shown to be regulated by transforming growth factor-β (TGF-β; Heimer et al., 1995). Decorin is an inhibitor of cell growth (Santra et al., 1995; DeLuca et al., 1996) and regulator of muscle cell responsiveness to TGF-β (Riquelme et al., 2001). TGF-β is a potent inhibitor of both proliferation and differentiation of myogenic cells (Allen and Boxhorn, 1987). A member of the TGF-β family, myostatin, has generated considerable interest in both the medical and agriculture research communities. Defects in the myostatin gene are responsible for dramatic increases in muscle mass seen in Belgian Blue and Piedmontese breeds of cattle (McPherron and Lee, 1997). Likewise, myostatin-null mice exhibit enhanced musculature (McPherron et al., 1997). Using a rodent model system to study atrophy, Srinivasan et al. (2004) demonstrated that levels of myostatin, the myostatin receptor activinR2b, and its downstream messenger cyclin-dependent kinase inhibitor p21 (CDKI p21) are elevated during muscular atrophy. As levels of myostatin are elevated during muscle

* Corresponding author. Tel.: +1 605 688 5431; fax: +1 605 688 6170.
E-mail address: Douglas.McFarland@sdstate.edu (D.C. McFarland).

1095-6433/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2006.04.020

breakdown, it is thought that this may serve to prevent premature satellite cell activation while degeneration occurs. When muscle regeneration commences, myostatin levels diminish (Kirk et al., 2000). In vitro studies have shown that myostatin inhibits cell proliferation, DNA synthesis, and protein synthesis but has no effect on protein degradation or apoptosis (Taylor et al., 2001). Administration of myostatin to explant cultures inhibits satellite cell activation and progression into the cell cycle (McCroskery et al., 2003). Although myostatin is principally found in skeletal muscle, it has also been localized to adipose tissue where it serves as a negative regulator of preadipocyte differentiation (Hirai et al., 2005).

In the present work, we have examined the effects of myostatin on the proliferation and differentiation of embryonic myoblasts and satellite cells derived from the turkey. To further examine the mechanism behind the effects of myostatin on cellular physiology, we have also investigated whether myostatin administration to myogenic cells influences the expression of decorin. It has recently been shown that decorin binds myostatin preventing its interaction with cellular receptors, thus serving as a modulator of this growth factor (Miura et al., 2006). This suggests that the mechanism of action of myostatin may be similar to that of TGF-β. In other work, Heimer et al. (1995) determined that TGF-β upregulates the synthesis of decorin. We, therefore, sought to determine if myostatin influenced the expression of decorin in avian myogenic cells.

The satellite cell clones utilized in this study were previously shown to differ in their responsiveness to growth factor stimuli (McFarland et al., 1995a; Yun et al., 1997). The fast growing cells (Early clones) were more responsive to the mitogenic effects of insulin-like growth factors, insulin, fibroblast growth factor, and platelet-derived growth factor compared to slow growing cells (Late clones). Likewise, the fast growing clones were more responsive to the proliferation and differentiation depressing effects of transforming growth factor-β.

2. Materials and methods

2.1. Materials

Embryonic myoblasts were isolated from the pectoral muscles of 15-day turkey (*Meleagris gallopavo*) embryos and cloned to remove fibroblast contamination as previously described (McFarland et al., 1991). Satellite cells were isolated and cloned from the pectoralis major muscles of one six-week-old Nicholas tom turkey as previously described (McFarland et al., 1995a; Yun et al., 1997). Satellite cells in the 6th and 7th passage and embryonic myoblasts in the 6th passage were used in these reported studies.

The composition of the defined medium is described in Table 1. Advanced Dulbecco's Modified Eagle Medium (Advanced-DMEM) and horse serum (HS), were obtained from Gibco-Invitrogen (Grand Island, NY, USA). Chicken serum (CS) was obtained from either Gibco-Invitrogen or Sigma-Aldrich Chemical Company (St. Louis, MO, USA). DMEM, antibiotic–antimycotic mixture, and gentamycin sulfate were obtained from Atlanta Biologicals (Norcross, GA, USA). Recombinant growth factors were purchased as follows: hepatocyte growth factor (HGF), R & D Systems (Minneapolis, MN, USA); fibroblast growth factor-2 (FGF-2) and insulin-like growth factor-I (IGF-I), PeproTech (Rocky Hill, NJ, USA); platelet-derived growth factor-BB (PDGF-BB), AUSTRAL Biologicals, (San Ramon, CA, USA); myostatin, Leinco Technologies, Inc. (St. Louis, MO) or PeproTech (Rocky Hill, NJ, USA). McCoy's 5A medium, insulin, and all other reagents, including fetuin, were purchased from Sigma. Cell cultureware were obtained from Greiner Bio-One, Inc. (Longwood, FL, USA). The South Dakota State University Animal Care and Use Committee approved all animal-handling procedures used in this study.

Table 1
Avian satellite cell serum-free medium composition *

Growth factors/hormones	
Platelet-derived growth factor-BB	5 ng/mL
Hepatocyte growth factor	1 ng/mL
Insulin-like growth factor-I	10 ng/mL
Fibroblast growth factor-2	20 ng/mL
Insulin	0.2 mg/mL
Vitamins	
Vitamin B_{12}	2 μg/mL
Biotin	0.2 μg/mL
Vitamin A (all-*trans*-Retinol)	0.286 μg/mL
Vitamin E (D-α-Tocopherol acid succinate)	0.129 μg/mL
Minerals	
$FeSO_4 \cdot 7H_2O$	4.98 μg/mL
$(NH_4)_6Mo_7O_{24} \cdot 4H_20$	1.24 ng/mL
$ZnSO_4 \cdot 7H_2O$	1.4 μg/mL
Nutrients and other components	
Fetuin (gel filtered)	0.2 mg/mL
L-glutamine	0.219 mg/mL
Linoleic acid (water soluble)	0.75 μg/mL
Thymidine	0.7266 μg/mL
Adenine	0.4053 μg/mL
Gelatin	10 ng/mL
Penicillin (base)	50 units/mL
Streptomycin (base)	50 μg/mL
Amphotericin B	0.125 μg/mL
Gentamycin sulfate	5 μg/mL

* Added to Advanced DMEM (GIBCO-Invitrogen Corp.).

2.2. Measurement of proliferation

For measurements of proliferation, cells were plated in plating medium (DMEM + 10% CS + 5% HS) in 24-well plates (10 replicates/treatment) and allowed to attach for 22 h. Cultures were then administered growth medium (McCoy's 5A medium + 10% CS + 5% HS) or defined medium (Table 1) ±20 ng/mL of myostatin daily. After 3 days of proliferation, wells were rinsed with PBS, blotted, and frozen for later analysis.

Proliferation was evaluated by determination of the DNA content of the wells using a fluorometric method previously described (McFarland et al., 1995b).

2.3. Measurement of differentiation

For measurements of differentiation rates, cells were grown in 24-well plates in growth medium until reaching 60% confluence (5 wells/treatment). Cells were then administered fusion medium (DMEM + 3% HS + 0.01 mg/mL gelatin + 1.0 mg/mL