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Periostin is temporally expressed as an extracellular matrix component in skeletal muscle regeneration and differentiation

Cansu Özdemir [a], Uğur Akpulat [a], Parisa Sharafi [a], Yılmaz Yıldız [a], İlyas Onbaşılar [b], Çetin Kocaefe [a,*]

[a] Dept. of Medical Biology, Hacettepe University School of Medicine, Ankara, Turkey
[b] Laboratory Animal Breeding and Research Unit, Hacettepe University School of Medicine, Ankara, Turkey

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ABSTRACT

The transcriptional events and pathways responsible for the acquisition of the myogenic phenotype during regeneration and myogenesis have been studied extensively. The modulators that shape the extracellular matrix in health and disease, however, are less understood. Understanding the components and pathways of this remodeling will aid the restoration of the architecture and prevent deterioration under pathological conditions such as fibrosis. Periostin, a matricellular protein associated with remodeling of the extracellular matrix and connective tissue architecture, is emerging in pathological conditions associated with fibrosis in adult life. Periostin also complicates fibrosis in degenerative skeletal muscle conditions such as dystrophies. This study primarily addresses the spatial and temporal involvement of periostin along skeletal muscle regeneration. In the acute skeletal muscle injury model that shows recovery without fibrosis, we show that periostin is rapidly disrupted along with the extensive necrosis and periostin mRNA is transiently upregulated during the myotube maturation. This expression is stringently initiated from the newly regenerating fibers. However, this observation is contrasting to a model that displays extensive fibrosis where upregulation of periostin expression is stable and confined to the fibrotic compartments of endomysial and perimysial space. In vitro myoblast differentiation further supports the claim that upregulation of periostin expression is a function of extracellular matrix remodeling during myofiber differentiation and maturation. We further seek to identify the expression kinetics of various periostin isoforms during the differentiation of rat and mouse myoblasts. Results depict that a singular periostin isoform dominated the rat muscle, contrasting to multiple isoforms in C2C12 myoblast cells. This study shows that periostin, a mediator with deleterious impact on conditions exhibiting fibrosis, is also produced and secreted by myoblasts and regenerating myofibers during architectural remodeling in the course of development and regeneration.

1. Introduction

Under physiological conditions, skeletal muscle exhibits a stable structure. However, in the case of injury, satellite cells – the resident somatic stem cells of the muscle – are activated to restore structural integrity (Charge and Rudnicki, 2004). Differentiation and repair both pursue cascade of transcriptional events harmonized by common myogenic regulatory factors (MRFs) and signaling pathways (Braun and Gautel, 2011). The transcriptional events responsible for the acquisition of the myogenic phenotype are relatively well understood. Repeated cycles of injury and regeneration impact the tissue architecture of the skeletal muscle, causing fibrosis, fatty infiltration and myofibrillary atrophy, which are the three hallmarks of chronic muscle degeneration. The molecular events that shape the extracellular matrix and cause fibrosis under pathological conditions, such as the dystrophies, aging or disuse atrophy are still obscure.

High throughput technologies provide insights into the transcriptional events that characterize differentiation, repair and adaptive response of the tissue. Using the open-access transcriptome data, we conducted an in silico survey to pinpoint a conjoint list of genes that were significantly altered in a set of transcriptome observations commonly targeting physiologic and pathologic conditions of skeletal muscle. These include time-course profiling of myoblast differentiation (Moran et al., 2002; Tomczak et al., 2004; Chen et al., 2006), various human skeletal muscle pathologies (Bakay et al., 2006), dystrophin-deficient (*mdx*) mouse diaphragm (Porter et al., 2004) and sarcopenia model in rat (Wang et al., 2012). This approach accentuated a number of commonly altered soluble or exported factors, including periostin. Periostin is a matricellular protein which is ubiquitously expressed during embryonic morphogenesis in the extracellular matrix and connective tissues. It is directly interacting with collagen and fibronectin during fibrillogenesis in early development (Kudo, 2011). In the postnatal life, periostin is abundant in connective tissues exposed to mechanical strains such as tendon, bone, heart valves and skin (Merle and

Abbreviations: MRFs, myogenic regulatory factors; DMEM, Dulbecco's Modified Eagle's Medium; FCS, fetal calf serum.

* Corresponding author at: Department of Medical Biology, Hacettepe University Faculty of Medicine, 06100 Sihhiye, Ankara, Turkey.
E-mail address: kocaefe@hacettepe.edu.tr (Ç. Kocaefe).

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Garnero, 2012). However, periostin upregulation is associated with fibrotic pathological conditions such as scar formation in wound healing, crash-induced bone damage, myocardial infarcts, pulmonary fibrosis, liver cirrhosis and fibrosis of the skeletal muscle (Hamilton, 2008; Rani et al., 2009; Dobaczewski et al., 2010; Merle and Garnero, 2012).

An attributed function promoting collagen cross-linking is the association of periostin to pathologic fibrotic events (Maruhashi et al., 2010). In skeletal muscle, periostin function is linked to fibrosis-related conditions such as strain injury evoked by eccentric exercise (Rani et al., 2009). Periostin knockout animals exhibit diminished fibroblastic proliferation in the course of cardiac recovery from myocardial infarction (Oka et al., 2007; Norris et al., 2009). Likewise, diminished fibrosis and enhanced myofiber regeneration are observed in knockout dystrophic mice (Lorts et al., 2012). Besides its role in chronic degenerative conditions, the abovementioned transcriptome results primed us to investigate the spatial and temporal expression of periostin in various models of muscle regeneration and myoblast differentiation.

2. Materials and methods

2.1. Animals and procedures

All experiments were conducted on three-month-old male Sprague–Dawley rats (225 g ± 30 g) and all procedures were performed according to institution-approved protocol and under strict biological containment (Approval Decision No.: 2009/30-4 and 2005/40-8).

The acute muscle injury model was accomplished via injection of cardiotoxin into the tibialis anterior (TA) muscles of adult rats. Briefly, following appropriate anesthesia and disinfection, 1 nmol of cardiotoxins (Sigma-Aldrich) was infiltrated using a Hamilton syringe. On the pre-determined days (1st, 3rd, 4th, 6th, 8th, 10th, and 14th) the time-course sampling was carried out. The total TA muscle from the right extremity was resected under general anesthesia prior to sacrifice ($n = 3$ for each time point). The extracted TA muscles were harvested and fresh frozen accordingly for RNA isolation, protein extraction or tissue sectioning.

Achilles tenotomy was used as a model to induce fibrosis in skeletal muscle. Following appropriate anesthesia and disinfection, partial resection of the Achilles tendon was performed on the right side by removing approximately 4 mm distal section. Soleus muscles from both the right and left extremities were resected under general anesthesia for a follow-up period of 6 weeks ($n = 3$ for each time point). The muscles were handled as previously described above.

2.2. Cells and in vitro studies

C2C12 mouse embryonic myoblast cells (Yaffe and Saxel, 1977) were used for the documentation of the periostin expression during proliferation, fusion and myotube maturation stages of differentiation. The C2C12 cell line was purchased from the Foot and Mouth Disease Institution's Animal Cell Culture Collection Facility (Ankara, Turkey) and all tissue culture consumables were purchased from Biochrom AG, Germany. Cells were maintained on "proliferation medium" which consisted of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (FCS), 2 mM L-Glutamine and 1% (w/v) streptomycin and penicillin. The medium was refreshed every 2 days. Upon reaching confluency, cells were induced for myogenic differentiation using a "differentiation medium," in which 2% horse serum was substituted for the 10% of FCS. Triplicated samples of 50% and 90% confluent cells under proliferation conditions in the 24th, 48th, and 96th hours following induction of differentiation were harvested using trizol reagent.

2.3. Isolation and in vitro differentiation of primary myoblasts

Expression kinetics of periostin was further documented in differentiating primary myoblasts (Kocaefe et al., 2010). Lower extremity muscles were dissected and subjected to collagenase type 1 (La Roche Ltd., Basel, Switzerland) and dispase (Sigma) digestion (0.5% w/v). Mononuclear cells were harvested following retrieval through a 30 μm mesh and Ficoll gradient and immediately plated on matrigel (BD Biosciences, NJ) coated tissue culture plates. Primary myoblasts were expanded in DMEM/Ham's Nutrient Mixture F12 medium (1:1) supplemented with 20% FCS and 2% Ultroser G (Pall, NY), 2 mM L-glutamine and 1% of penicillin and streptomycin, and differentiated in a similar medium where the FCS and Ultroser G supplements were replaced with 2% horse serum. Total RNA from primary myoblast cultures proliferating at 50% and 90% confluency and differentiated for the 6th, 24th, 48th, 72nd and 96th hours was collected using trizol.

2.4. RNA isolation and qPCR studies

The time-course periostin mRNA expression levels were investigated by reverse transcriptase coupled quantitative real-time PCR (qPCR). 50–75 mg of TA muscle was rapidly disrupted using a bead-beater and the RNA extraction was accomplished using Trizol reagent upon the manufacturer's recommendations. RNA integrity and quality were assessed via denaturing agarose gel electrophoresis and UV absorbance measurements. 1 μg of total RNA was reverse transcribed into cDNA using Improm II (Promega) reverse transcriptase following manufacturer's protocols. The quantitative gene expression analysis was accomplished using the SYBR Green technique. Equal amounts of cDNA were used for the real-time amplification of the target transcripts using Jumpstart SYBR Green mix in 3 mM final Mg^{++} concentration (Sigma-Aldrich) according to the manufacturer's recommendations on a Rotorgene 6000 (Corbett Life Science, Australia) fluorometric PCR instrument. Following an initial denaturation of 2 min at 94° C, two-step PCR reaction was conducted for 40 cycles that consisted of 5 s at 94 °C for denaturation and 20 s at 60° C for annealing and elongation. Fluorimetric acquisition on green channel was done at the end of the annealing and elongation step. The sequences of primer pairs are provided in Table 1. The amplification of periostin cDNA was accomplished using a primer pair matching both rat and mouse species spanning exons 16 and 23 (Ensembl Exon accession numbers for mouse and rat are ENSMUSE00000172742, ENSMUSE00000804649 and ENSRNOE00000121871, ENSRNOE00000340025, respectively). This approach yielded the amplification of the six putative alternatively spliced exons. The expression of β-actin was used to normalize the periostin expressions. The relative expressions were further normalized to the expression of the control samples and the results were presented as relative fold changes. The one-way ANOVA test was utilized to test the significance of the periostin expression ($p > 0.05$). PCR products were run on 3% agarose gels to verify the absence of any non-specific products and primer dimers, and alternatively spliced isoforms were documented. A densitometric analysis was performed on agarose gel images using ImageJ software (http://imagej.nih.gov/ij/) (Schneider et al., 2012). The C2C12 and rat skeletal muscle cDNA amplicons were further analyzed using cycle sequencing with Applied Biosystems big dye terminator 3.1 reagents on an ABI 3130 genetic analyzer. In order to determine the splice variants in mouse C2C12 cells, full length cDNA products were also cloned and sequenced using primers targeting 5′UTR and stop codon.

2.5. Immunostaining and histochemistry

Tissue samples were sectioned to 8 μm thickness and investigated by standard immunostaining methods for periostin immunolocalization. Primary anti-developmental type myosin heavy chain (Leica, NLC-MHCd corresponding to Myh3 isoform, working dilution 1:50) and