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SMHS1 is involved in oxidative/glycolytic-energy metabolism balance of muscle fibers

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Abstract

With the aim of finding important mediators of muscle atrophy, we cloned SMHS1, a novel gene that was found to be upregulated in rat soleus muscle atrophied by restriction of activity. The SMHS1 amino acid sequence shares 65% similarity with RTP801—which is a cellular stress response protein regulated by HIF-1—but SMHS1 expression was demonstrated to be independent of HIF-1. SMHS1 was found to be mainly expressed in skeletal muscle, and comparisons of its expression in atrophied versus hypertrophied muscles and in oxidative versus glycolytic muscles suggested that SMHS1 contributes to the muscle energy metabolism phenotypes.

Keywords: Skeletal muscle; Atrophy; Hypertrophy; Muscle fiber type; RTP801; HIF-1

The well-documented plasticity of skeletal muscle is largely based on the heterogeneity of fiber types which vary in metabolic equipment, contraction speed, and fatigability [1–3]. Briefly, at one end of the classification, slow-twitch oxidative fibers (type I) have a slow contraction time and are fatigue resistant—they are responsible for both maintaining posture and carrying out slow repetitive movements. Then, we found fast-twitch oxidative fibers (type IIa) with a rapid contraction time associated to fatigue resistance. The other end of the classification includes fast-twitch glycolytic fibers (types IIx and IIb), which have a fast contraction time and low fatigue resistance. They are adapted for high-power output. In addition of these principal fibers, there exist several intermediary and transitory fibers (types I/IIa, IIa/IIx, and IIx/IIb). Chronic skeletal muscle activity variations cause changes in muscle mass and metabolism, which are associated with shifts in fiber types [4–6]. These well-established changes ultimately modify muscle performance, e.g., oxidative slow- to glycolytic fast-twitch transitions in the case of atrophy of slow-twitch muscles (soleus muscle). Many cellular components are involved in this switch, including myofibril protein isoforms, oxidative enzymes, glycolytic enzymes, calcium homeostasis players, transcription factors, and signaling molecules [7–10]. Several previous studies, including those conducted by our laboratory, focused on large-scale gene profiling or proteomic analysis in experimental rat and mouse models of muscle atrophy induced by hindlimb unweighting or immobilization [11–16]. In order to identify genes involved in skeletal muscle adaptive responses to chronic activity modifications, we characterized a novel gene that we previously demonstrated to be twofold upregulated in rat soleus muscles atrophied by restriction of activity imposed by hindlimb

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suspension (03A12 clone) [12]. This gene, named SMHS1 (skeletal muscle hindlimb suspension 1), encodes a protein with 65% similarity (35% identity) to the RTP801 protein [17]. RTP801 is a novel recently identified protein under the control of hypoxia-inducible factor 1 (HIF-1). After we submitted the full-length SMHS1 cDNA sequence to GenBank, the same cDNA sequence was found through in silico EST contigization and named RTP801-like [17].

In this study, we cloned SMHS1 full-length cDNA and characterized the SMHS1 sequence and expression, especially in different muscle mass and energy metabolism conditions. Our data suggest that SMHS1 is a novel gene potentially important for the oxidative versus glycolytic specificities of muscle fibers.

Materials and methods

Animals and tissues. All procedures were approved by the Local Centre National de la Recherche Scientifique Ethics Committee. Ten-week-old Ico: OF1 (Caw) animals were used for mouse tissue RNA preparations. Atrophied soleus and gastrocnemius muscles were derived from a previous study [12]. Ten-week-old female Sprague–Dawley rats ($n = 9$/experimental group) were used for soleus muscle compensatory hypertrophies and glycolytic versus oxidative muscle analysis. For hypertrophy experiments, rats were anesthetized and separated in two groups. The first group was sham-operated for control, and the second group was submitted to synergist gastrocnemius muscle ablations to induce a compensatory hypertrophy of plantaris and soleus muscles by overload. Six weeks later, rats were sacrificed and soleus muscles were taken, weighted, and immediately frozen in liquid nitrogen.

Cell culture. C_2C_{12} mouse skeletal muscle cells (ATCC No. CRL-1772) were grown in Dulbecco's modified Eagle's medium with 4.5 g/L glucose and 10% fetal calf serum. Myotube C_2C_{12} differentiation was induced by withdrawing fetal calf serum and adding 10 μg/ml insulin, 5 μg/ml transferrin, and 2% horse serum.

cDNA library screening and cloning of rat SMHS1 full-length cDNA. An oligo(dT)-random primed cDNA library of rat skeletal muscle (Clontech) was screened with the 03A12 cDNA fragment [12]. Approximately 1.2×10^6 λgt11 phage clones were transfected into host strain Y1090r- and then transferred onto nylon membranes (Macherey-Nagel) for hybridization, as previously described [12]. Positive clones were plaque-purified and the cDNA phage inserts were PCR-purified using λgt11 specific vector amplimers.

RNA analysis. Multiple rat tissue RNA blots were purchased from Clontech Laboratories. Total RNA was prepared with RNAInstaPure kit (Eurogentec). Northern blot analyses were performed as previously described, with ^{32}P-labeled mouse partial SMHS1 or actin cDNA and 18S rRNA probes [12]. Reverse transcription reactions were carried out with M-MLV-RT (Invitrogen) and oligo(dT). Advantage Klen *Taq* polymerase (Clontech) was used for PCR amplification of partial SMHS1 cDNA with the forward primer 5′-GAAACAGAGCCGTTG ACCAT-3′ and the reverse primer 5′-ATTAGCCACTCATTAGGG AC-3′. The 18S rRNA cDNA fragment was amplified with the forward primer 5′-AGTTGGTGGAGCGATTTGTC-3′ and the reverse primer 5′-GGCCTCACTAAACCATCCAA-3′. PCR samples were taken in exponential amplification phase, i.e., 25 cycles for SMHS1 and 20 cycles for 18S rRNA. Agarose electrophoretic bands and Northern blot specific signals were quantified using the PCBas software package.

In situ hybridizations were performed on 12 μm serial sections of mouse total embryos, at 75 °C overnight, with 239 bp anti-sense and 400 bp sense SMHS1 digoxigenin–UTP-labeled riboprobes. Riboprobes were synthesized with the DIG RNA labeling kit (Roche), from SMHS1 cDNA cloned in pGEM-T Easy (Promega) using SP6 and T7 transcription starts after plasmid linearization with *Nhe*I. Visualization of digoxigenin (DIG)-nucleic acids with alkaline-phosphatase-conjugated anti-DIG antibody was carried out according to Roche's procedure.

Protein analysis. Western blot analyses were performed on protein whole extracts of wild-type C2C12 cells as previously described [18]. Anti-HIF-1α monoclonal antibody (AbCam, clone hif$^{\alpha 67}$) and anti-FLAG monoclonal antibody (Sigma) were used at 1:200 and 1:2000 dilution, respectively.

Statistical analysis. *t* test was performed using SigmaStat software in order to compare the differences between two groups of values. Statistical differences were noted ($^*P < 0.05$).

Results

Molecular cloning and sequence analysis of SMHS1

We previously cloned the 03A12 novel partial cDNA on the basis of its upregulation in rat soleus muscle atrophied after activity restriction induced by hindlimb suspension [12]. This cDNA was used to screen a rat skeletal muscle cDNA library, and a putative 1.1 kb full-length cDNA was cloned and named SMHS1 for "skeletal muscle hindlimb suspension 1." The nucleotide sequence (GenBank Accession No. AF327511) contained a 579 bp open reading frame compatible with the size of the ~1.2 kb SMHS1 mRNA. The mouse ortholog cDNA (GenBank Accession No. AF327512) was cloned through RT-PCR experiments using rat SMHS1 oligonucleotides as primers and mouse muscle RNAs as templates. The open reading frame accuracy was confirmed by expression in C2C12 muscle cells (not shown). The highest SMHS1 sequence homologies were found with a few vertebrate proteins, including an unknown zebrafish protein and a hypothetical *Xenopus tropicalis* protein (Fig. 1). The only meaningful sequence homology concerned the 65% amino acid residue similarity (35% identity) with a recently identified protein named RTP801 [17], or REDD1 [19], or dig2 [20]. This protein is involved in cellular stress responses and its RNA transcription is regulated by hypoxia-inducible factor 1 (HIF-1). Although no known functional domains were found within the sequences reported in Fig. 1, it was interesting to note the presence of several residue stretches highly identical in the different proteins, especially in the C-terminal portion, suggesting that these domains have been conserved throughout evolution.

SMHS1 mRNA expression

Hybridization of rat RNAs with the rat SMHS1 cDNA revealed an abundant ~1.2 kb mRNA with high expression in skeletal muscle, in addition to expression