Characterization of Porcine Satellite Cells

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Abstract— **Lean growth rate in most domestic animals differs with muscle type. Satellite cells (SC) potentiate muscle growth but little is known regarding how populations of SC differ with muscle type, especially in pigs. Therefore, the objective of this study was to characterize SC from red (RST) and white (WST) portions of the semitendinosus muscle and determine their capacity to proliferate, differentiate and express various myosin heavy chain (MyHC) isoforms *in vitro*. Porcine satellite cells were isolated from RST and WST muscles of 6-week-old piglets and cultured under standard muscle culture conditions. RST yielded more (P<0.001) SC per gram muscle compared to WST, $8.1x10^4$ cells versus $6.7x10^4$ cells/gram muscle, respectively. Satellite cells from RST proliferated faster (P<0.001) than those from WST, as indicated by a shorter cell doubling time, 18.6 h versus 21.3 h, respectfully. As a result, SC from RST formed myotubes quicker than those from WST. Once differentiated, however, SC from WST differentiated faster (P<0.05) than those from RST in the first 24 h, 41.6% versus 34.3%, respectively; but reached similar ultimate fusion percentages by 48 h. Over 90% of MyHC expressed in fully differentiated SC from both RST and WST was restricted to the embryonic isoform. Type IIA MyHC mRNA was detected at low levels, while type IIX MyHC mRNA was not detectable. Even so however, myotube cultures from RST expressed more (P<0.01) type I MyHC isoform mRNA than those from WST, whereas those cultures from WST expressed more (P<0.05) type IIB MyHC transcripts. These data show SC from red and white muscles differ and suggest intrinsic characteristics of these cells may be partially restricted to a particular muscle type prior to birth.**

Keywords— **Porcine, Satellite Cell, Myosin heavy chain**

I. INTRODUCTION

Meat production depends on skeletal muscle growth is largely affected by muscle fiber type composition. Satellite cells (SC), first discovered in frog muscle by Mauro in 1961, are the postnatal source of nuclei contributed to growing muscle fibers [1]. The muscle hypertrophy occurs through proliferation of satellite cells followed by differentiation and fusion into existing muscle fibers [2].

Skeletal muscle fiber types are classified based on their myosin heavy chain (MyHC), contractile activity, and metabolic properties [3]. Muscle fibers containing MyHC type I are slow twitch and oxidative fibers, that are rich in mitochondria and myoglobin; while type II fibers, including IIA, IIX and IIB, are fast twitch fibers and have more glycolytic metabolism [4]. Skeletal muscle collectively is a dynamic tissue composed of different types of fibers that ensure diverse functions.

It has been proposed that fiber type diversity derived from cultured SCs is limited by intrinsic differences in the differentiation of satellite cells [5]. However, cultured SCs from rabbit slow muscle differentiate into myotubes expressing slow as well as neonatal and fast MyHC isoforms. This suggests that there is heterogeneity within SC pools and may be differentially pre-programmed in a muscle-specific manner [6].

To date, little is known about functional and phenotypic relationship of SCs and their muscle origin in pig skeletal muscle. Therefore, the objective of this study was to characterize SC from red (RST) and white (WST) portions of the semitendinosus muscle (ST), and determine their capacity to proliferate, differentiate and express various myosin heavy chain (MyHC) isoforms *in vitro*.

II. MATERIALS AND METHODS

Porcine Satellite Cell Isolation and Cell Culture

Satellite cells were isolated from ST muscles as previously described [7]. Briefly, ten mg of RST and WST muscle sample were minced and digested in PBS

containing 0.8 mg of protease / mL. After enzymatic digestion, cell supensions were filtered and cells were harvested by centrifugation at 1,200g 15 min. Satellite cell isolates were plated on 2% matrigel coated 6-well plates in proliferation medium consisting of MEM supplemented with 10% fetal bovine serum with antibiotics. Cells were kept at 37℃ in a humidified incubator with 5% CO_2. When cells reached 80% confluence, they were induced to differentiate by culturing in differentiation medium containing 2% horse serum.

Proliferation and differentiation Index

Satellite cell proliferation was measured every 24 h for 4 days using the Promega Non-Radioactive Cell Proliferation Assay Kit (Madison, Wisconsin, USA). Briefly, RST and WST satellite cells were plated on 96-well matrigel-coated plates at 10,000 cells per cm^2 and incubated for 24 h. An aliquot (15 µl) of dye solution was added to cultures and left for 1 h before the addition of 100 µl of stop/solubilization solution. The OD of each well was measured at 570 nm after an overnight incubation.

Differentiation was measured by staining the nuclei with giemsa or DAPI solution. Fusion percentage was calculated by dividing the myotube nuclei with the number of total nuclei. A myotube was defined as three or more nuclei within a cell membrane.

Determination of cell yield

Cell yield was expressed as the number of cells released per gram muscle wet weight. Minced muscle samples were weighed before enzymatic digestion. After isolation, cells were seeded on 6-well plates and allowed to attach for 24 h. Cells were harvested with trypsin and were counted with a hemocytometer. Cell yield was calculated by comparing the total number of cells with the weight of the muscle sample.

Population doubling time

RST and WST derived satellite cells were seeded in matrigel-coated, 24-well culture plates at 10,000 cells per cm^2. After attachment, the medium was changed and this was considered 0 h for the proliferation experiment. Incubation was continued for 2 d, and at 0, 12, 24, 36, and 48 h, three wells of satellite cells were harvested using trypsin and counted using a hemocytometer. Cell doubling time was then calculated by online software (www.doubling-time.com).

Quantitative RT-PCR

After three days of differentiation, RNA was isolated using TRIzol reagent following the manufacturer's instructions (Invitrogen, San Diego, CA). Briefly, 1 ml TRIzol reagent was applied on each well and total RNA was isolated and quantified. First-strand cDNA was synthesized from 3 ug of total RNA using random hexanucleotide-primed cDNA synthesis. Quantitative Real time PCR was performed on ABI 7500 Fast Real Time PCR System (Applied Biosystems, Foster City, CA). Each PCR reaction contained 200 ng of cDNA, 10 pmol primers, and iQ

Table 1. Nucleotide sequences of the primers used for quantitative reverse transcription-PCR

Gene name	Sequence		Accession no.
β-Actin	Forward	CGA CAA CGG CTC CGG CAT GT	U07786
	Reverse	CAT CAC GCC CTG GTG TCG GG	
MyHC Embryonic	Forward	CCC GGC TTT GGT CTG ATT T	Da Costa 2002
	Reverse	GGT GTC GGC TGC GAG TCA CA	
MyHC I	Forward	GGC CCC GGC CAG CTT GA	L10129
	Reverse	TGG CTG CGC CTT GGT TT	
MyHC IIA	Forward	CTT CCA GGC TGC ATC TTC TC	U11772
	Reverse	TTC CCT CCT TCT CTG CTC TG	
MyHC IIX	Forward	CTA TTT TTG GGG AGG CTG CT	U90719
	Reverse	TTC CCT CCT TCT CTG CTC TG	
MyHC IIB	Forward	CAC TTT AAG TAG TTG TCT GCC TTG AG	U90720
	Reverse	GGC AGC AGG GCA CTA GAT GT	

SYBR Green Supermix in a total volume of 10 ul. Primers used in qPCR are listed in Table 1.

Statistics

The statistical analysis of data was analyzed with JMP using Student's T test. The level of significance was set at $P < 0.05$.

III. RESULTS

Cell yield of muscle samples

Cell yield largely depended on animal age and enzyme efficiency. In this experiment, we strictly controlled all the conditions in order to compare RST and WST muscle cell yield. RST muscle samples yielded more ($P < 0.001$) SCs per gram muscle than WST ($8.1x10^4$ cells versus $6.7x10^4$ cells/gram muscle, respectively).

Proliferation rate

The cell proliferation assay demonstrated that satellite cells isolated from RST proliferated faster ($P<0.001$) than those from WST (Fig. 1) as indicated by a shorter cell doubling time, 18.6 h versus 21.3 h, respectfully.

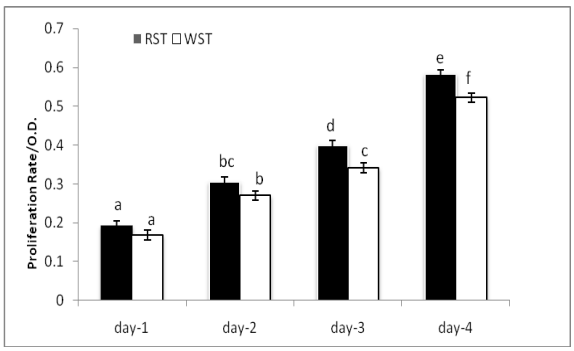

Figure 1. Proliferation rates of RST and WST derived satellite cells. Data were measured at day-1, day-2, day-3 and day-4. Results are means ± SE. Means bearing different labels differ ($P < 0.05$).

Satellite cell fusion rates

In the myogenic differentiation experiment, the same amount of satellite cells were seeded on 6-well plates ($2.5 x10^5$ cells per well, enough for inducing differentiation immediately after cells attached to plates which excluded the influence of different proliferation rate of RST and WST SC). Our results revealed that once differentiated, SC from WST differentiated faster ($P < 0.05$) than those from RST in the first 24 h, 41.6% versus 34.3%, respectively; but reached similar ultimate fusion percentages by 48 h (Fig.2).

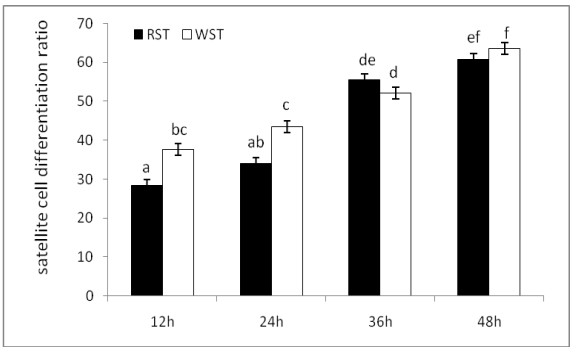

Figure 2. Differentiation rates of RST and WST derived SC over time. Differentiating cells were fixed and stained at 12 h, 24 h, 36 h and 48 h in culture. Means ± SE bearing different labels differ ($P < 0.05$).

Myosin heavy chain expression in SC culture

In fully differentiated SC cultures from both RST and WST, myotubes rarely expressed adult MyHC. Over 90% of MyHC expressed was restricted to the embryonic isoform (Fig. 3). There was no significant difference in embryonic MyHC expression between RST and WST. Type IIA or IIX MyHC mRNA was not detectable. Myotubes from RST culture expressed more ($P < 0.01$) type I MyHC isoform mRNA than those from WST, whereas those cultures from WST expressed more ($P < 0.05$) type IIB MyHC transcripts.

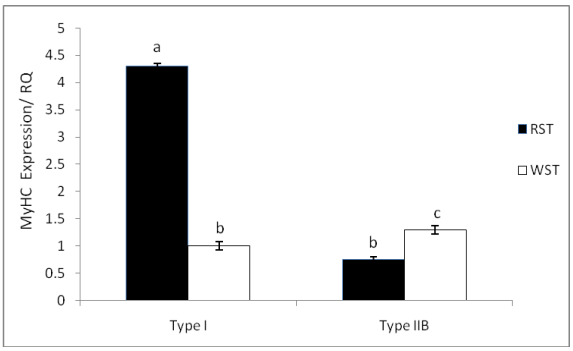

Figure 3. Adult MyHC type I and IIB mRNA expression in fully differentiated SCs from RST and WST. Means ± SE bearing different labels differ ($P < 0.05$).

IV. DISCUSSION

In this study, we characterized SCs from RST and WST. Higher cell yields and faster differentiaon rates for cultured SCs from RST suggest great turnover and SC inclusion in red muscles

The ultimate differentiation rates of RST and WST were identical. Due to the lack of nerve and endocrine signals, *in vitro* cell culture can not completely represent *in vivo* muscle growth and development. Further investigation is needed to explore the effect of nerve and endocritic signal on SC differentiation.

The present data showed that the majority of MyHC expression in SC culture appeared to be restricted to the embryonic isoform. Myotubes derived from RST expressed more type I and WST derived SC expressed more type IIB. Therefore, our data suggest that SCs are pre-programmed in their muscle of origin. However, the question of whether red and white muscle derived SCs can grow and differentiate into red and white muscle fibers, respectively, may largely depend on how these embryonic myotubes grow and differentiate into an adult phenotype.

In conclusion, the present results show that SC from red and white muscles differ and suggest intrinsic characteristics of these cells may be partially restricted to a particular muscle type.

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