Expression of sialyltransferases from the ST3Gal, ST6Gal and ST6GalNAc families in mouse skeletal muscle and mouse C2C12 myotube cell cultures

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Research Article

Keywords: C2C12 myotubes, sialylation, sialyltransferases, skeletal muscles

Posted Date: March 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-316783/v1

Abstract

I. Background. In skeletal muscles the sialic acids have a great significance for their functional maintenance and proper structural organization. Our work for the first time described the expressions of ST3Gal, ST6Gal and ST6GalNAc sialyltransferases specific for glycoproteins in mouse skeletal muscles and murine C2C12 myotube cell cultures.

II. Methods and Results. Lectin histochemistry, cytochemistry and lectin blot were used to demonstrate the membrane localization and the electrophoretic profiles of α-2,3- and α-2,6-sialylated glycoproteins. The expression levels of sialyltransferases were analyzed by real time RT-PCR and western blot. The enzymes ST6Gal2 and ST6GalNAc1 were not expressed in skeletal muscle tissue and C2C12 myotubes. In both experimental groups mRNAs of the ST3Gal family prevailed over the mRNA expressions of the ST6Gal and ST6GalNAc families. The profiles of STR expressions showed differences between the two experimental groups, illustrated by the absence of expressions of the mRNA for the ST3Gal6 and ST3GalNAc3 enzymes in the C2C12 cell samples and by the different shares of the enzymes ST3Gal3 and ST3Gal4 in both experimental groups. The different patterns of enzyme expressions in both experimental groups corresponded with differences between their α-2,3- and α-2,6-sialylated glycoprotein profiles.

III. Conclusions. These results could be a useful addendum to the knowledge concerning the glycosylation of the skeletal muscle tissue. In addition, this report would be helpful and informative for any researches in future where the C2C12 myotube cell cultures will take a place as an experimental model.

Introduction

The attachment of monosaccharide residues is one of the most complicated co- or posttranslational modifications that proteins can undergo, resulting in an abundant, diverse, and highly regulated repertoire of cellular glycans. Two major classes of oligosaccharides are defined according to the nature of the linkage between the carbohydrate chain and the polypeptide region. An O-glycan (O-linked oligosaccharide) is usually bound to the polypeptide via N-acetylgalactosamine (GalNAc) to a serine (Ser) or threonine (Thr) residue and can be extended into a variety of different structural core classes. An N-glycan (N-linked oligosaccharide) is a sugar chain covalently linked to an asparagine (Asn) residue of a polypeptide chain within the consensus peptide sequence: Asn-X-Ser/Thr [1, 2].

One of the most fascinating building units of the oligosaccharide constructions are the sialic acids. They represent a family of over 40 modifications of the N-acetylneuraminic acid (Neu5Ac). The sialic acids typically occupy the terminal position of the glycoconjugate sugar chains, usually via α-2,3-, α-2,6-, or α -2,8- glycosidic bond [3-5]. The glycosidic bonds are generated by highly specific enzymes that belong to four sialyltransferase families. The members of the families beta-galactoside alpha-2,3-sialyltransferase (ST3Gal), beta-galactoside alpha-2,6-sialyltransferase (ST6Gal) and N-acetylgalactosaminide alpha-2,6-

sialyltransferase (ST6GalNAc) are widely spread in different tissues, while the enzymes from the alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase family (ST8SiA) are mostly expressed in the brain [6]. The sialylation of glycoproteins or glycolipids always occurs into the Golgi and afterwards they are transported to the cell membrane. Because of their terminal position on the oligosaccharide chains, the sialic acids participate in almost all types of recognition phenomena and adhesion mechanisms [7, 8].

In skeletal muscles the sialic acids are very important for the functional maintenance of glycoproteins involved in fiber structure and neuromuscular junctions, development and regeneration, muscle excitability and exercise performance [9-14].

Even if the sialylation is not as much abundant as in other tissues, the muscles are very sensitive to sialic acid deficiency due to mutations, which results in a variety of diseases with a sever and progressive loss of motility as a common feature [15, 16]. Histological expressions of sialylated glycoproteins in adult human skeletal muscles were already described in details [17]. By now however, the only identified sialylated glycoprotein in skeletal muscles is the α-dystroglycan, a member of the dystrophin-associated glycoprotein complex [18]. Comprehensive information about the expression of enzymes from the sialyltransferase families is also missing in the available literature.

The aim of this work was to investigate the sialylation in mouse skeletal muscle tissue and C2C12 mouse myotube cell culture in the aspect of localization of α-2,3- and α-2,6-sialylated glycoproteins, relative quantification of sialyltransferase expressions and comparison of the α-2,3- and α-2,6-sialylated glycoprotein profiles.

Materials And Methods

Ethical procedures

All animal experiments were performed in compliance of Regulation № 20/01.11.2012 on the minimum requirements for protection and welfare of experimental animals and the requirements for the sites for their use, breeding and / or delivery, issued by the Ministry of Agriculture and Food of Republic of Bulgaria.

Mouse tissue samples collection

Five male white laboratory mice, 6-8 weeks old, were humanly euthanized. Tissue specimens were excised from the femoral and gluteal muscles and fixed with freshly prepared methacarn fixative [19], or stored at -80°C for further studies. Specimens from lungs, spleen, brain, liver, intestine, colon and kidneys were archived in low temperatures, too. After processing, the fixed specimens were embedded in paraffin.

Cell cultures

C2C12 mouse myoblast cell line (ATCC® CRL-1772™) was purchased from LGC Standards USA (Manchester, NH, USA). The cells were cultured for 48h in Dulbecco's Modified Eagle's Medium (DMEM,

Sigma-Aldrich, St. Louis, MO, USA) with a high glucose content of 4.0 g / L supplemented with 10% fetal bovine serum (FBS, Gibco-Thermo Fisher Scientific, Waltham, MA, USA), penicillin 100 IU / ml and streptomycin 100 µg / ml, (AppliChem, Darmstadt, Germany) into plastic tissue culture flasks (Orange Scientific, Braine-l`Alleu, Belgium) or onto 12 mm oval glass cover slips (Glaswarenfabrik Karl Hecht GmbH, Sondheim, Germany), until 90% confluence of the monolayer was achieved. Further differentiation into myotubes was induced by changing the growth medium to differentiation medium – Dulbecco's Modified Eagle's Medium, supplemented with 2% horse serum (Sigma-Aldrich). The cells were dissociated by 0.05% solution of trypsin (Gibco-Thermo Fisher Scientific) with 0.025% ethylenediaminetetraacetic acid (AppliChem) and counted with an automatic cell counter (Countess™, Invitrogen™, ThermoFisher Scientific). Samples with approximate concentration of $5x10^6$ cells/ml were stored at -80°C for further molecular and proteomic studies. The myotube layers onto the cover slips (Glaswarenfabrik Karl Hecht GmbH, Sondheim, Germany) were submitted for lectin cytochemistry.

Lectin histo- and cytochemistry

Cover slips with myotube cultures and skeletal muscle tissue sections were treated with biotinylated lectins – *Maackia amurensis* lectin (MAL, Vector Laboratories, Burlingame, CA, USA), specific for α-2,3-sialic acids [20] and *Sambucus nigra* agglutinin (SNA, Vector Laboratories), specific for α-2,6-bound sialic acids [21]. The tissue sections were first rehydrated, and the myotubes were treated with 0.3% Triton in buffer. The further steps were performed in dark. All samples were incubated for 30 min with 1 µg/mL methanol solution of 4`6-diamidino-2-phenylindole (DAPI, AppliChem, Darmstadt, Germany), then with SNA or MAL (1 µg/mL) for 60 min, and finally with Streptavidin-FITC (1:100, Sigma-Aldrich) for 30 min. Control samples were treated with buffer instead of lectins. The samples were mounted in Vectashield mounting medium (Vector Laboratories) and observed with light microscope Leica DM 5000B (Leica Camera AG, Wetzlar, Germany) under UV, blue, green and UV/violet fluorescent filters. The obtained parallel images of each sample were merged using ImageJ Software [NIH, USA, 22].

Gene expression analyses

The experiments described in this section were designed to evaluate the expression of mRNA of sialyltransferases ST3Gal1, 2, 3, 4 and 6, ST6Gal1 and 2, and ST6GalNAc1, 2, 3 and 4 in mouse skeletal muscle tissue samples and mouse C2C12 myotube cell cultures. The levels of expressions were estimated via normalization versus the expressions of peptidyl prolyl isomerase A (PPIA) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as reference genes. All primers were designed using the NCBI Blast Tool [23] in a way to span at least one intron sequence. The full names of investigated genes, the primer sequences and the size of the amplified products are available as a supplementary file. The oligonucleotides were purchased from HVD Biotech Vertriebs (Vienna, Austria). The substrate specificities of all sialyltransferases analyzed in this study are shown in Table 1.

Five skeletal muscle tissue samples with approximate weight of 30 mg each and five aliquots of C2C12 myotube cell cultures from different passages with approximate concentration of $5x10^6$ cells/ml were

homogenized using Tissue Ruptur II homogenizer (Qiagen, Hilden, Germany) on ice. Total RNA was isolated and purified by GeneMatrix Universal RNA Purification Kit (EurX®, Gdansk, Poland), strictly following the corresponding protocols recommended by the producer. The yield and purity of the collected RNA were measured using S-300 Spectrophotometer (Boeco, Hamburg, Germany).

Approximately 2 μg total RNA from each sample were used for first strand cDNA synthesis. The RT Master Mix contained 5x Reaction Buffer, 20 U RiboLock Rnase Inhibitor, 1 mM dNTPs, 100 pmol Random hexamer primers, 200 U RevertAid Reverse Transcriptase (all of them Fermentas of Thermo Fisher Scientific) and DEPC-treated water (Sigma-Aldrich). The reaction mixture was first incubated at room temperature for 10 min, then at 42°C for 1h, and the reaction was terminated at 70°C for 10 min. The generated cDNA was quantified and the samples were stored at -80°C.

Real-time PCR was designed on approximately 700 ng cDNA as a template in 20 μl total volume of reaction using SG qPCR Master Mix (2x), 0.25 U uracyl-N-glycolase (UNG), nuclease free water (all from EurX) and 0.2 μM R- and F-primers specific for amplification of fragments of GAPDH, PPIA, ST3Gal1, 2, 3, 4 and 6, ST6Gal 1 and 2, and ST6GalNAc1, 2, 3 and 4. Three real-time PCR reactions/sample in duplicate were performed for amplification of each fragment of interest using 36-well RotorGene™ 6000 Real-time Analyzer (Corbett Life Science-Qiagen).

The data were analyzed using Rotor Gene Q Series Software (Qiagen) and the relative quantification of the STR expressions was calculated by the ΔΔCt method [26] versus PPIA and GAPDH as reference genes. After each run, a High Resolution Melting Curve Analysis (HRM) was performed to verify the specificity of the amplified products, which were visualized on 2.5% agarose gel supplemented with Simply Safe nucleic acid stain (EurX) versus 100-1000 bp DNA Ladder (EurX) and the gels were photographed with a gel documentation system Vision (Scie-Plas Ltd, Cambridge, UK).

Statistical analysis of the gene expression quantification

Statistical analysis of the data was performed using GraphPad Prism 5.03 software (San Diego, CA, USA). One Way Anova analysis with test of Bonferroni was computed to detect statistically significant differences between the Ct values of the qPCR products, and the results were interpreted as follows: $P < 0.001$ = highly significant, $P < 0.01$ = very significant, $P < 0.05$ = significant.

SDS-PAGE, lectin- and western blotting

Skeletal muscle tissue samples with an approximate weight of 30 mg each and aliquots of C2C12 myotube cell cultures from different passages with an approximate concentration of $5x10^6$ cells/ml were homogenized in 0.6M Tris buffer, containing 150 mM NaCl, 5 mM EDTA and 1% CHAPS (all purchased from Sigma-Aldrich), supplemented with Proteinase inhibitor cocktail, set 3 (Sigma-Aldrich), using Tissue Ruptur II homogenizer (Qiagen) on ice, and then centrifuged at 21000 x g, for 1 h at 4°C. The supernatants were used for methanol/chloroform protein precipitation as described by Fic et al. [27]. The protein pellet was reconstituted in 6 M Urea buffer, containing 1.5 M Thiourea, 3% CHAPS, and 66 mM

DTT (all purchased from Sigma-Aldrich), and stored at -20°C. The protein content was measured by the method of Bradford [28] on spectrophotometer S-300 (Boeco).

Approximately 30 µg from each sample were mixed with 4xLoading buffer (EurX), samples were heated at 98°C for 10 min and were then loaded on 10% polyacrylamide gel. SDS-PAGE was performed under reducing conditions as described by Laemmly [29].

Gels were then stained with colloidal coomassie brilliant blue [30], or were forwarded to western blotting on 0.45 µm nitrocellulose membranes (Sigma-Aldrich), as described by Towbin et al. [31]. The membranes designated for lectin-affino blots were blocked with 5% non-fat dry milk (Sigma-Aldrich) for 1 h, then incubated with biotinylated SNA (Vector Laboratories) or MAL (Vector Laboratories) (1 µg/mL) for 1 h, and finally treated with streptavidin horseradish peroxidase (HRP, Vector Laboratories) for 30 min at room T°C. The membranes designated for western blots were treated with goat blocking serum (Vector Laboratories) for 1 h, then incubated with rabbit antibodies against GAPDH (1:2000, Thermo Fisher Scientific), ST3Gal6 (1µl/mL) and ST6GalNAc3 (2µl/mL) (Sigma-Aldrich) for 2 h, and finally treated with WestVision Peroxidase Polymer Anti-Rabbit IgG (Vector Laboratories) for 30 min at room T°C. The color reaction on all membranes was developed after exposure to DAB Peroxidase Substrate solution (Vector Laboratories). The approximate molecular weight of the detected protein bands was estimated versus Perfect™ Tricolor Protein Ladder (EurX), ranging from 11 to 245 kDa.

Results

Localization and protein profiles of α-2,3- and α-2,6-sialylated glycoproteins

The lectin histochemistry and cytochemistry in our study demonstrated the membrane localization of α-2,3- and α-2,6-sialylated glycoproteins in mouse skeletal muscle samples and the C2C12 cell line (Fig. 1A). The cell line samples were much more reactive towards MAL in comparison with SNA. Both experimental groups showed similar protein profiles with a slight difference between the patterns of the α-2,3-sialylated glycoproteins, demonstrated by MAL-afiino blot. The C2C12 cell culture samples showed a higher number of α-2,6-sialylated glycoproteins, as demonstrated by the SNA-affino blot. In both experimental groups, the lectin affino blots revealed sialylated glycoproteins with an approximate molecular weight between 120 and 15 kDa (Fig 1B).

Expression of sialyltransferases

Our study was designed to analyze the expressions of members from the β-galactoside α-2,3-sialyltransferase (ST3Gal), β-galactoside α-2,6-sialyltransferase (ST6Gal) and GalNAc α-2,6-sialyltransferase (ST6GalNAc) families, operating on glycoproteins (Takashima 2008), which substrate preferences were described in Table 1.

The specificity of the primers used in the study was evident by the single peaks of the melting curves indicating a single product of amplification (data not shown). The enzymes ST6Gal2 and ST6GalNAc1

didn`t show products of amplification in the skeletal muscle samples and in the C2C12 cell line (Fig. 2). Expressions of mRNAs for all the rest of the sialyltransferases were detected in mouse skeletal muscle samples (Fig. 3A). Expressions of mRNA for the enzymes ST3Gal6 and ST6GalNAc3 were not detected in the C2C12 muscle cell samples (Fig. 3B) and this was confirmed also by protein western blot (Fig. 3C).

According to the percent distribution analysis of the expressions of investigated sialyltransferases in both experimental groups (Fig. 4), mRNAs of the ST3Gal family prevailed over the mRNA expressions of the ST6Gal and ST6GalNAc families. The profiles of STR expressions were different between skeletal muscle tissue samples and C2C12 cell cultures, illustrated by the missing expressions of the mRNA for the ST3Gal6 and ST3GalNAc3 enzymes in the C2C12 cell samples and by the different shares of the enzymes ST3Gal3 and ST3Gal4 in both experimental groups. Among the members of the ST6GalNAc family, the expression of ST6GalNAc4 enzyme prevailed strongly in both experimental groups. The expression of the ST6GalNAc2 sialyltransferase was also significantly lower in the C2C12 myotubes.

Discussion

Apart from the broad knowledge about the extracellular proteoglycan components and their role in the muscle growth and development [32], most of the information concerning glycosylation of the skeletal muscle tissue is related to inherited disease states [33, 34] and actually very little is known about its normal glycoproteome.

As already mentioned, the only sialylated glycoprotein discovered in the skeletal muscle tissue by now, was the α-dystroglycan bearing α-2,3-linked sialic acid residues [18]. Our results showed however the presence of at least several α-2,3- and α-2,6-sialylated glycoproteins, still not identified.

A very important aspect in this scientific topic is the expression of sialyltransferases in muscles. The great variety of the oligosaccharide constructions used as acceptors by the sialyltransferases predetermines the diversity of these enzymes, which were grouped into four families according to the glycosidic linkages they synthesize. From amino acid sequence similarities, substrate specificities and gene structures, the members of each sialyltransferase family were classified into subfamilies [6].

In mice and humans, ST6Gal2 is one of the two members of the ST6Gal family. Both members utilize the Gal-β-1,4-GlcNAc structure on glycoproteins and oligosaccharides as acceptor substrates. The ST6Gal1 gene has a wide range of tissue expression, however the ST6Gal2 gene is expressed in a stage-specific (embryonic stage) and a tissue-specific (adult brain) manner [35], as confirmed also by our results.

The enzymes ST6GalNac1 and ST6GalNAc2 were classified into a common subfamily of the ST6GalNAc family. Both enzymes exhibit similar substrate specificity, utilizing GalNAc- (Tn antigen), Gal-β-1,3-GalNAc- (T antigen) and SiA-α-2,3-Gal-β-1,3-GalNAc- (sialyl-T antigen) structures on O-glycans of glycoproteins as acceptor substrates [36]. However, ST6GalNAc1 was reported as a major sialyl-Tn synthase, whereas the ST6GalNAc2 acts preferentially on T antigen [24]. In our study, we observed a

positive signal of amplification of ST6GalNAc1 specific product in mouse lung tissue, but not in our experimental muscle and myotube samples.

ST3Gal6 is a member of the ST3Gal family and utilizes preferentially the Gal-β-1,4-GlcNAc structure on glycoproteins and glycolipids as an acceptor substrate [37]. ST6GalNAc3 together with ST6GalNAc4 are classified into a common subfamily from the corresponding sialyltransferase family. These two enzymes utilize the SiA-α2,3-Gal-β-1,3-GalNAc (sialyl-T antigen) structure on glycoproteins as an acceptor substrate [38]. Another intriguing difference between the muscle tissue and the cell culture are the shares of the enzymes ST3Gal3 and ST3Gal4, which belong to the same subfamily of α-2,3-sialyltransferases. These two enzymes utilize the same oligosaccharide structures as substrate acceptors but with quite opposite preferences [25].

Herein we report the absence of expression of the ST3Gal6 and ST6GalNAc3 genes in C2C12 mouse myotube cell culture, as well as the different patterns of expression of ST3Gal and ST6GalNAc sialyltransferases between mouse skeletal muscle tissue and the cell culture. Since this study had been performed for the first time, we cannot comment these findings any further and more profound research is necessary to elucidate its biological meaning.

The development of new technologies in life sciences opened in the late 1980s a new division of molecular biology named “glycobiology”. Since then, a huge knowledge was accumulated concerning the chemistry of carbohydrates, the enzymology of glycan biosynthesis and degradation, the structure of glycoconjugates, the recognition of glycans by specific proteins and the roles that the glycans occupy in complex biological systems. In this rapidly growing field in the natural sciences however, the skeletal muscles remained somehow not quite well explored object of investigation. The different patterns of enzyme expressions between them corresponded with differences between their α-2,3- and α-2,6-sialylated glycoprotein profiles. These results could be a useful addendum to the knowledge concerning the glycosylation of the skeletal muscle tissue. In addition, this report would be helpful and informative for any researches in future where the C2C12 myotube cell cultures will take a place as an experimental model.

Declarations

Acknowledgements

This work was financially supported by the Bulgarian National Science Fund with Grant DN01/16.

Declarations

Funding

This work was financially supported by the Bulgarian National Science Fund with Grant DN01/16.

Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Availability of data and material

All data are available under request.

Code availability

Not applicable.

Authors` contributions

RM conceived of the study, designed the experiments and the primers, performed the molecular and proteomic experiments and drafted the manuscript. AG carried out the cell culturing and counting. KT participated with the histological experiments. SP supervised the work, performed the statistical analyses and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval

All animal experiments were performed in compliance of Regulation № 20/01.11.2012 on the minimum requirements for protection and welfare of experimental animals and the requirements for the sites for their use, breeding and / or delivery, issued by the Ministry of Agriculture and Food of Republic of Bulgaria.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

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Tables

Table 1. Substrate specificity of the sialyltransferases, operating on glycoproteins [24, 25], investigated in this study. The monosaccharides in bold indicate a residue onto which a sialic acid is transferred via α-2,3- or α-2,6-glycosidic linkage. Gal – galactose, GalNAc – N-acetyl-D-galactosamine, GlcNAc – N-acetyl-D-glucosamine, SiA – sialic acid, Ser – serine, Thr – threonine.

β-Galactoside-α-2,3-sialyltransferase family (ST3Gal)	
ST3Gal1	**Gal**-β-1,3-GalNAc
ST3Gal2	**Gal**-β-1,3-GalNAc
ST3Gal3	**Gal**-β-1,3-GlcNAc > **Gal**- β-1,4-GlcNAc > **Gal**-β-1,3-GalNAc
ST3Gal4	**Gal**-β-1,3-GalNAc > **Gal**- β-1,4-GlcNAc > **Gal**-β-1,3-GlcNAc
ST6Gal6	**Gal**- β-1,4-GlcNAc > **Gal**-β-1,3-GlcNAc
β-Galactoside-α-2,6-sialyltransferase family (ST6Gal)	
ST6Gal1	**Gal**- β-1,4-GlcNAc
ST6Gal2	**Gal**- β-1,4-GlcNAc
GalNAc α-2,6-sialyltransferase family (ST6GalNAc)	
ST6GalNAc1	**GalNAc**-α-1-Ser/Thr (Tn Ag) > Gal-β-1,3-**GalNAc**-α-1-Ser/Thr (T Ag)
	SiA-α-2,3-Gal-β-1,3-**GalNAc**-α-1-Ser/Thr (sialyl-T Ag)
	Gal-β-1,3-**GalNAc**-α-1-Ser/Thr > **GalNAc**-α-1-Ser/Thr
ST6GalNAc2	SiA-α-2,3-Gal-β-1,3-**GalNAc**-α-1-Ser/Thr
	SiA-α-2,3-Gal-β-1,3-**GalNAc**-
ST6GalNAc3	SiA-α-2,3-Gal-β-1,3-**GalNAc**-
ST6GalNAc4	

Figures

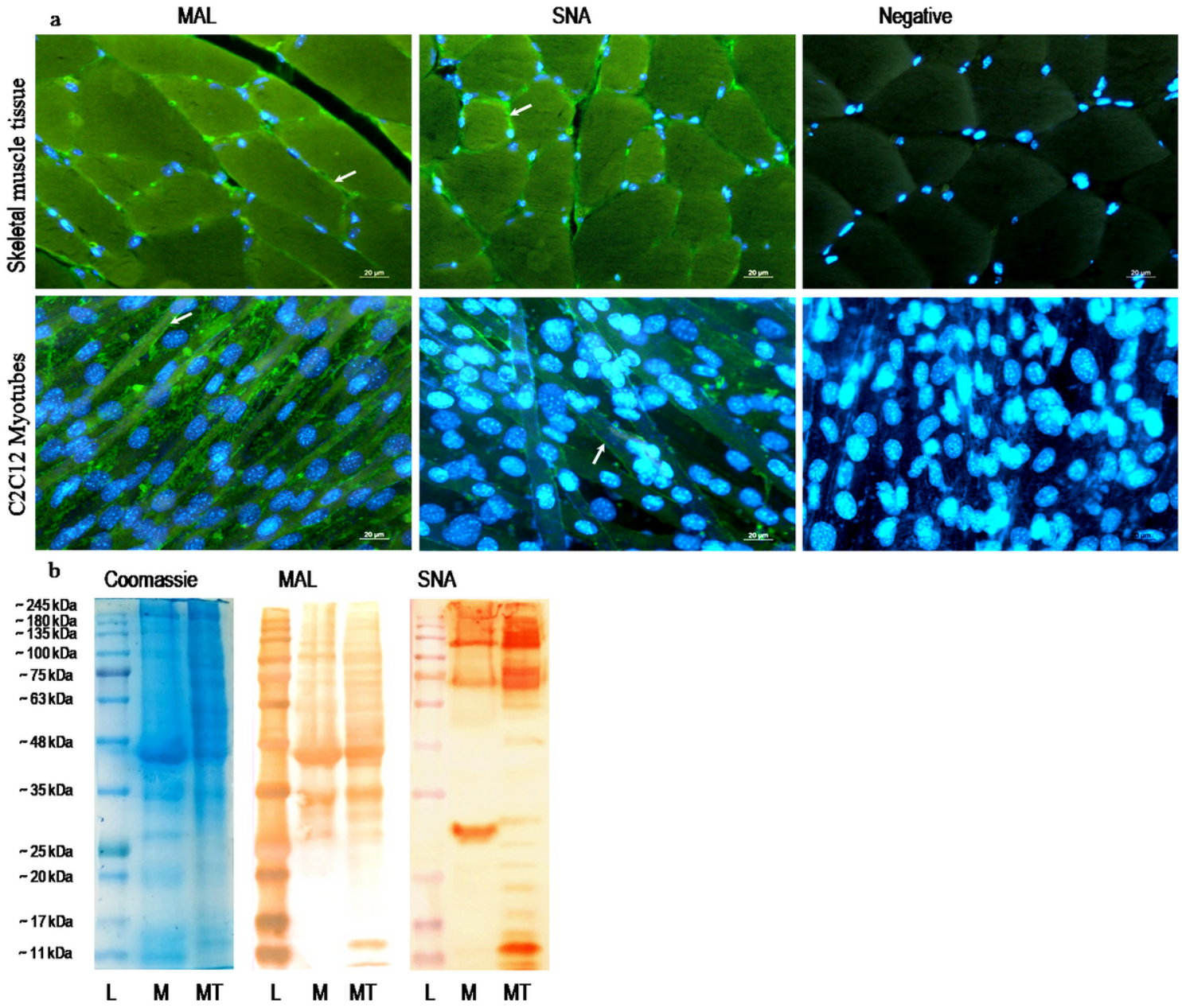

Figure 1

Sialylated glycorpoteins in mouse skeletal muscle tissue and mouse C2C12 myotubes. a - Skeletal muscle tissue sections and C2C12 myotube cultures were stained with lectins MAL and SNA specifically recognizing α-2,3- and α-2,6-sialylated glycoproteins located on the cell membranes (arrows). Streptavidin-FITC, DAPI. b - SDS-PAGE, MAL and SNA lectin affino-blot of mouse skeletal muscle tissue (M) and C2C12 myotube samples (MT), loaded on 10% gels versus Perfect™ Tricolor Protein Ladder (EurX) ranging from 11 to 245 kDa (line L), showing different patterns of sialylation. Streptavidin-HRP, DAB.

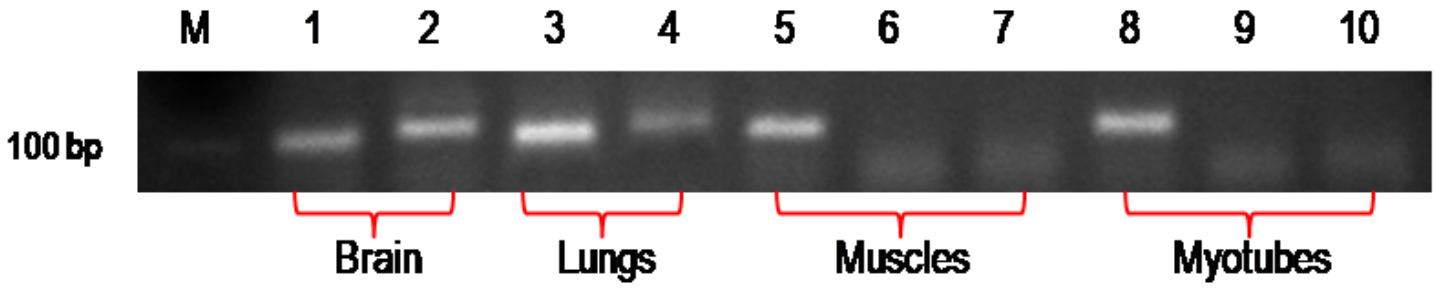

Figure 2

Absence of amplification products specific for mouse ST6Gal2 and ST6GalNAc1 sialyltransferases in mouse skeletal muscles and C2C12 myotubes. Mouse brain and lungs were used as positive expression controls. Lanes: M – 100 bp fragment of DNA Ladder, 1 and 2 – GAPDH (103 bp) and ST6Gal 2 (115 bp) expressions in brain, 3 and 4 – GAPDH and ST6GalNAc1 (117 bp) expressions in mouse lungs, 5, 6 and 7 – GAPDH, ST6Gal2 and ST6GalNAc1 expressions in mouse skeletal muscles, 8, 9 and 10 – GAPDH, ST6Gal2 and ST6GalNAc1 expressions in C2C12 myotubes.

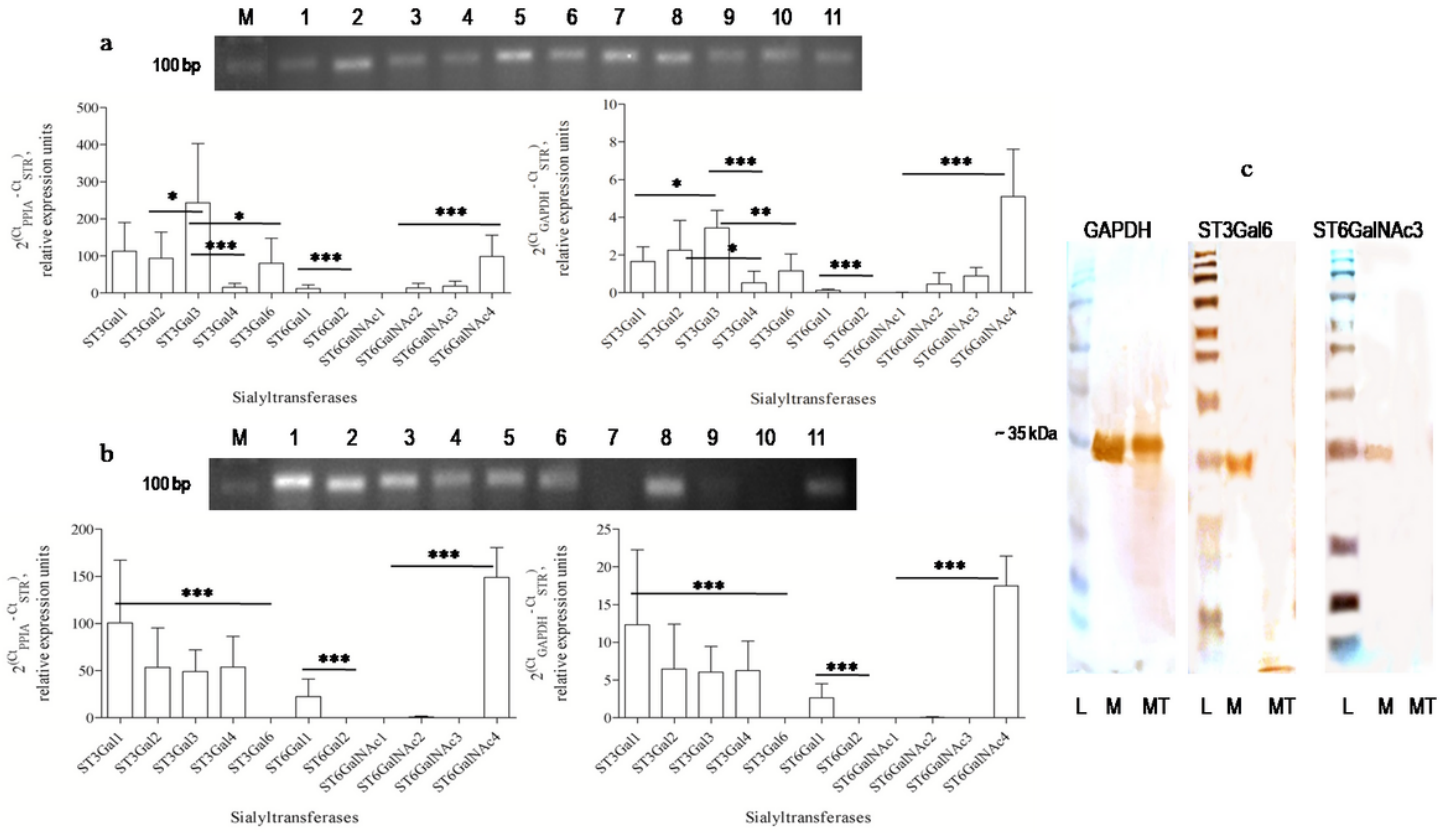

Figure 3

Expressions of mouse sialyltransferases analyzed by real time RT-PCR in skeletal muscle tissue (a) and C2C12 myotube cultures (b), and by western blot (c). Panels a and b – The photographs show amplification products specific for mouse STR on 2.5% agarose gel: M – 100 bp fragment of DNA Ladder, 1 – PPIA (115 bp), 2 – GAPDH (103 bp), 3 – ST3Gal1 (112 bp), 4 – ST3Gal2 (116 bp), 5 – ST3Gal3 (118

bp), 6 – ST3Gal4 (119 bp), 7 – ST3Gal6 (112 bp), 8 – ST6Gal1 (107 bp), 9 – ST6GalNAc2 (117 bp), 10 – ST6GalNAc3 (117 bp), 11 – ST6GalNAc4 (116 bp). The charts represent a relative quantification of STR expressions calculated by the ΔΔCt method versus PPIA (left) and GAPDH (right) as reference genes from six individual samples in triplicate. The bars show the standard deviation. The stars indicate statistically significant difference between sialyltransferase expressions in each family: *** P < 0.001, ** P< 0.01, * P< 0.05. Panel c – Western blots of mouse skeletal muscle tissue (M) and C2C12 myotube samples (MT), with polyclonal rabbit antobodies against GAPDH ThermoFisher Scientific), ST3Gal6 and ST6GalNAc3 (Sigma-Aldrich) sialyltransferases, loaded on 10% gels versus Perfect™ Tricolor Protein Ladder (EurX) ranging from 11 to 245 kDa (line L), showing absence of expression of both enzymes by the C2C12 myotubes. ImPress™ HRP Anti-Rabbit IgG, DAB.

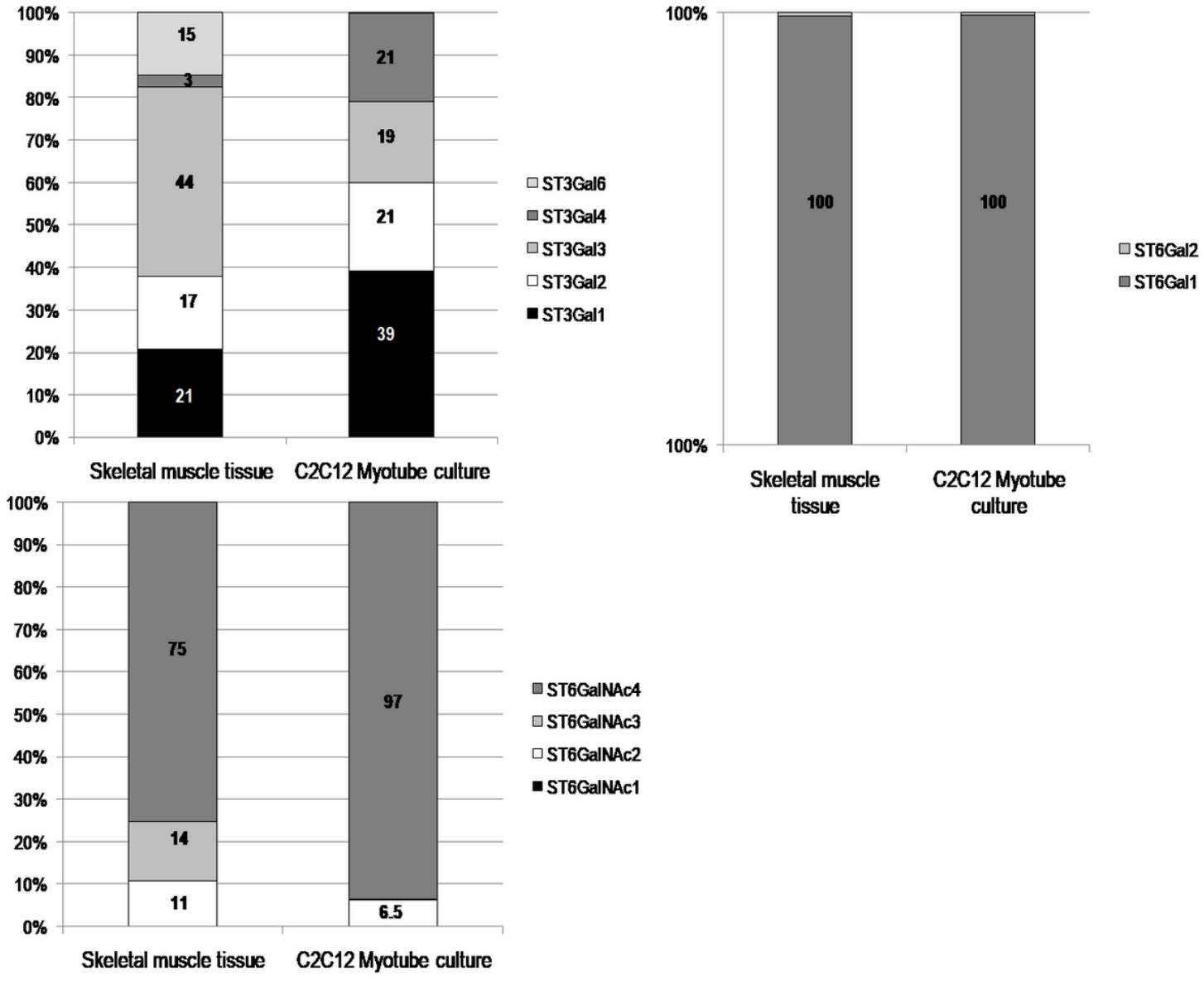

Figure 4

Percent distribution of the expressions of the enzymes sialyltransferases from the ST3Gal, ST6Gal and ST6GalNAc families in mouse skeletal muscle and mouse C2C12 myotubes. Normalization versus PPIA.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- RositsaProfilingSTRSupplementary.docx