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Sarcoglycan are not muscle-specific: hypothetical roles

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Summary

The sarcoglycan complex is a multimember transmembrane complex interacting with other proteins to provide a mechano-signaling connection from the cytoskeleton to the extracellular matrix in myofibers. This complex plays a key role at the membrane and is crucial in maintaining sarcolemma viability in muscle fibers. Recent observations have demonstrated that in the lung this glycoprotein is associated with both alveoli and bronchioles, and that the urogenital and digestive tracts are ε-sarcoglycan positive. Further addressing this issue, in this work we extend our previous studies to better verify whether the sarcoglycan complex also exists in epithelial tissue. All our observations showed staining for all sarcoglycans to be a normal pattern in all tested epithelial cells. We hypothesize a key role for sarcoglycans in bidirectional signaling between cells and extracellular matrix, and an important role in the regulation of inhibitory synapses and of blood brain barrier.

Key words

Sarcoglycan, skeletal muscle, smooth muscle, epithelial cells

Introduction

The sarcoglycan complex (SGC) is a multimember transmembrane complex interacting with other members of the dystrophin-glycoprotein complex (DGC) to provide a mechano-signaling connection from the cytoskeleton to the extracellular matrix in myofibers (Campbell 1995; Liu and Engvall 1999). SGC plays a key role at the cell membrane and is crucial in maintaining sarcolemma viability in muscle fibers (Liu and Engvall 1999). Moreover, sarcoglycans (SGs) may be important in maintaining proper calcium ion balance or may support the development and maintenance of muscle cells through signaling functions (Wheeler and McNally 2003).

The SGC consists of four transmembrane proteins: α-SG, specifically expressed in skeletal and cardiac muscle; β-SG, most abundant in cardiac and skeletal muscle but also expressed in placenta, kidney, liver, and lung (Bönnemann et al. 1995); and γ- and δ-SG, that are highly similar among themselves and similar to β-SG.

Whereas δ-SG is detected in all types of muscle, γ-SG is expressed exclusively in striated muscle. Attempts to immunolocalize γ-SG in smooth muscle have failed, and the question of whether γ-SG or a smooth muscle isoform exist is unanswered, although previous reports have anticipated the presence of γ-SG in smooth muscle (Durbeej et al. 2000).

A fifth sarcoglycan subunit, ε-SG, is more broadly expressed, showing a wider tissue distribution (Ettinger et al. 1997). Despite its homology to α-SG and its presence

in skeletal muscle, endogenous ε-SG is unable to rescue phenotypes associated with α-SG loss.

Recently, a novel mammalian sarcoglycan, the ζ-SG, has been identified by an antibody specific to δ-SG. This protein is a protein highly related to γ- and δ-SG and is reduced at the cell membrane in muscular dystrophy. ζ-SG was also found as a component of the vascular smooth muscle sarcoglycan complex and to present a costamer pattern (Wheeler et al. 2002).

Previous data showed that ε-SG immunoreactivity, like β- and δ-SG, was also widely distributed in non-muscle tissues. Recent observations have demonstrated that in the lung this glycoprotein was associated with both alveoli and bronchioles, and that the urogenital and digestive tracts were ε-SG positive (Ettinger et al. 1997).

Addressing this issue, in this work we extend our previous studies, performing immunofluorescence investigations to better verify whether the SGC also exists in epithelial tissue. In particular, we performed an immunofluorescence analysis of samples of normal human epithelia obtained from the gastrointestinal, urogenital, vascular, and respiratory tracts. Moreover, we performed a preliminary analysis on biopsies obtained from cerebral and renal tissue, in order to demonstrate that sarcoglycan can be non muscle-specific.

Materials and Methods

Biopsies were obtained from 10 male patients who underwent surgery but who were not affected by any pathology regarding epithelial cells. Patients were between 30 and 60 years of age. We obtained biopsies from gastrointestinal, urinary, respiratory, and cerebral tissue. All patients gave informed consent and all procedures were in accordance with the Helsinki Declaration of 1975.

Biopsies were fixed in 3% paraformaldehyde in a 0.2 M phosphate buffer, pH 7.4, for 2 hr. After numerous rinses in 0.2 M phosphate buffer and PBS, the biopsies were infiltrated with 12% and 18% sucrose to obtain a gradual substitution of saline solution with glucose solution and avoid disruption of cell membranes during successive phases. Finally, sections were frozen in liquid nitrogen. Twenty-μm-thick sections were incubated with primary antibodies for 2 hr. The following primary antibodies obtained from Novocastra Laboratories (Newcastle upon Tyne, UK) were used: mouse monoclonal anti-α-sarcoglycan (diluted 1:100), mouse monoclonal anti-β-sarcoglycan (diluted 1:200), mouse monoclonal anti-γ-sarcoglycan (diluted 1:100), mouse monoclonal anti-δ-sarcoglycan (diluted 1:50), and mouse monoclonal anti-ε-sarcoglycan (diluted 1:100). In order to define the distribution of proteins, we utilized anti-GFAP (1:100; Santa Cruz Biotecnology, Inc) to detect astrocyte cells and SMI32 (1:1000; Covance) to identify a subset of pyramidal neurons in cortex. In all reactions, TRITC-conjugated IgG anti-mouse in goat was used as the first fluorochrome (1:100 dilution; Jackson ImmunoResearch Laboratories, West Grove, PA) and applied for 1 hr after incubation with the primary antibody. Sections were then observed and photographed using a Zeiss LSM 510 confocal microscope (Carl Zeiss; Jena, Germany), equipped with an argon laser (458, 488 λ) and two HeNe lasers (543 and 633 λ). All images were digitalized at a resolution of 8 bits into an array of 2048 x 2048 pixels. Optical sections of fluorescent specimens were obtained using HeNe laser (543 nm)

and argon laser (458 nm) at a 1-min, 2-sec scanning speed with up to eight averages; 1.50-μm-thick sections were obtained using a pinhole of 250. Contrast and brightness were established by examining the most brightly labeled pixels and choosing settings that allowed clear visualization of structural details while keeping the highest pixel intensities close to 200. Digital images were cropped and figure montages prepared using Adobe Photoshop 5.0 (Adobe Systems; Palo Alto, CA).

Results

By confocal laser scanning microscopic observations, we studied the immunostaining patterns of α-, β-, γ-, δ-, and ε-SGs using specific antibodies. Indirect immunofluorescence applied to epithelial cells of the respiratory tract revealed the presence of all five tested SGs, both in nasal epithelium (Fig. 1A-E) and in bronchial epithelium (Fig. 1F-J).

In the observations on the urinary tract we showed that α-, γ-, and δ-SG (Fig. 2A-C) were shown normally by immunofluorescence; also in the digestive tract (Fig. 2D-E) α- and δ-SG showed a normal staining pattern. Moreover, all other sarcoglycans were present in all tested epithelial cell types (data not shown). Our observations on renal tissue revealed immunofluorescence for all SGs (Fig. 2F-J) to be normal in this tissue.

In cerebral tissue we performed triple-localization reactions for SG, SMI-32 and DAPI, in order to evidence the pyramidal neuron. Our observation showed normal immunofluorescence of α- (Fig. 3A) and γ-SG (Fig. 3B); other SGs showed a similar immunofluorescence (data not shown). In order to evidence the presence of SGs in glial cells also, we performed triple-localization reaction between SG, GFAP and DAPI. Our results showed the presence of all SGs, in this case including ε-SGs (Fig. 3C), in glial cells also.

Discussion

We carried out a semiquantitative study on SGC using normal human samples of epithelial tissue. We found the simultaneous expression of six molecules of this family and hence hypothesize a presence of all SGs also in non-muscle tissue. Initially, the sarcoglycans were considered as a complex of four transmembrane proteins (α, β, γ, and δ) primarily expressed in skeletal muscle (Bönnemann et al. 1995) and closely associated with dystrophin and the dystroglycans in the muscle membrane (Suzuki et al. 1992). A critical question is whether the SGC exists in muscle fibers exclusively, which would exclude a key role for them in other tissues.

In our previous immunohistochemical and molecular investigations carried out on surgical biopsies of human adult visceral smooth muscle, we showed that these SGs all coexist in the same fiber. Based on these findings, we hypothesized the presence of a pentameric structure of SGC and not a conventional heterotetrameric unit (Anastasi et al., 2005; Anastasi et al., 2007). By immunohistochemistry, studying smooth muscle fibers in proximity to the mucosa, our observations have revealed a presence of SGs in epithelial cells also. In this way, our attention has been drawn to this region, because the SGC always had been considered specific of muscle fibers.

Previous data have showed that ε-SG immunoreactivity, like that for β- and δ-SGs, was also widely distributed in non-muscle tissues (Ettinger et al. 1997). Recent observations have demonstrated that, in the lung, this glycoprotein is associated with both alveoli and bronchioles and that the urogenital and digestive tracts are ε-SG positive (Ettinger et al. 1997).

To investigate this question, we carried out an immunofluorescence study on α-, β-, γ- and δ- and ε-subunits of SGs, analyzing epithelial cells of digestive, respiratory, urinary and cerebral tissue, in order to check if other sarcoglycans also are present in non-muscle tissue.

All our observations have shown a normal staining patterns for all SGs in all tested epithelial cells. On this basis we can hypothesize novel roles for SGC. First of all, our results allow to confirm that SGC plays a key role in bidirectional signaling between cells and extracellular matrix, mediating all signals with external environment. Moreover, we can propose additional roles of SGC for the cerebral tissue. In this tissue, an intriguing hypothesis is that SGs could play an important role in the regulation of inhibitory synapses and of blood brain barrier. These data provide the first suggestion that SG are not only muscle-specific and may give way to new approaches for the individuation of the causes of more pathologies.

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Figures

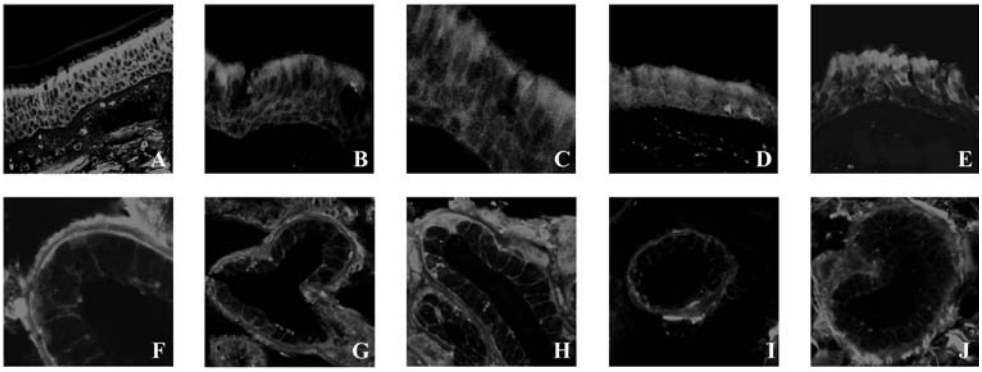

Fig. 1: Single-localization reaction in epithelial cells of the respiratory tract revealed a normal pattern for all five tested sarcoglycans, both in nasal epithelium (A-E) and in bronchial epithelium (F-J).

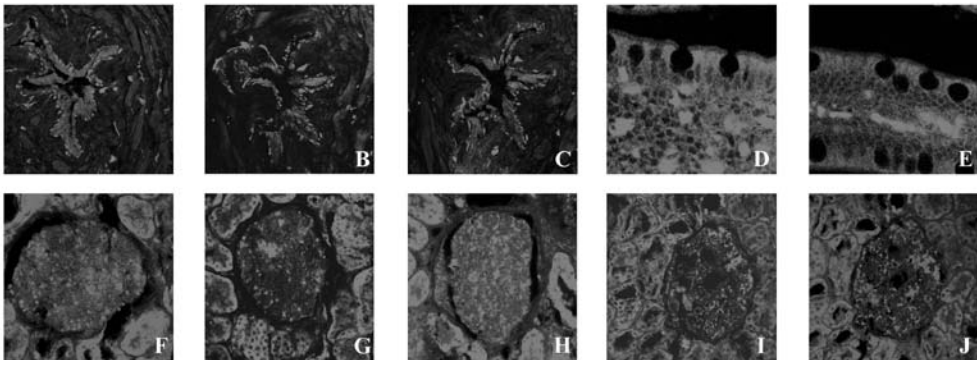

Fig. 2: In the urinary tract also α-, γ-, and δ-sarcoglycan showed normal immunofluorescence staining (A-C); the same behaviour was observable in the digestive tract (D-E). In renal tissue, all single reaction slides showed a normal fluorescence of all sarcoglycans (F-J).

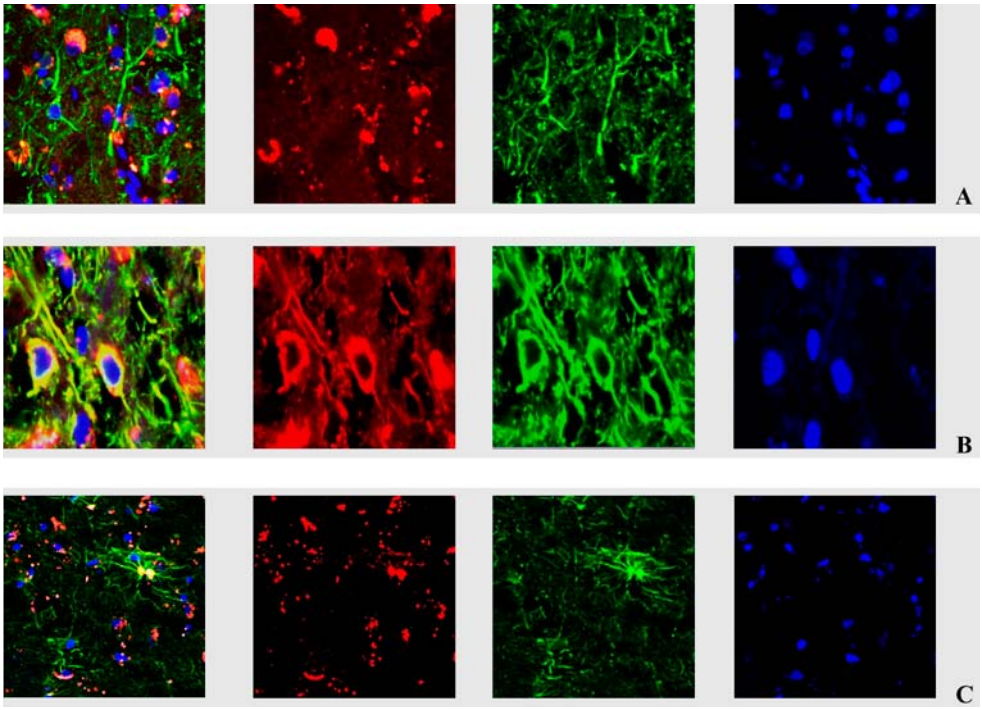

Fig. 3: Triple-localization reactions in cerebral tissue of α-sarcoglycan (red), SMI-32 (green) and DAPI (blu) (A), and of γ-sarcoglycans (red), SMI-32 (green) and DAPI (blu) (B). In all observation tested sarcoglycan showed a normal staining. Also in triple-localization reaction of ε-sarcoglycan (red), GFAP (green), DAPI (blu) sarcoglycans are present (C). From left to right, in the panels: triple staining, red staining, green staining, blue staining.