Commitment of Satellite Cells Expressing the Calcium Channel α2δ1 Subunit to the Muscle Lineage

Tammy Tamayo, Liliana Grajales, and Jesús García

Department of Physiology and Biophysics and Center for Cardiovascular Research, University of Illinois at Chicago, 835 South Wolcott Avenue, Chicago, IL 60612, USA

Correspondence should be addressed to Jesús García, garmar@uic.edu

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Satellite cells can maintain or repair muscle because they possess stem cell properties, making them a valuable option for cell therapy. However, cell transplants into skeletal muscle of patients with muscular dystrophy are limited by donor cell attachment, migration, and survival in the host tissue. Cells used for therapy are selected based on specific markers present in the plasma membrane. Although many markers have been identified, there is a need to find a marker that is expressed at different states in satellite cells, activated, quiescent, or differentiated cell. Furthermore, the marker has to be present in human tissue. Recently we reported that the plasma membrane α2δ1 protein is involved in cell attachment and migration in myoblasts. The α2δ1 subunit forms a part of the L-type voltage-dependent calcium channel in adult skeletal muscle. We found that the α2δ1 subunit is expressed in the majority of newly isolated satellite cells and that it appears earlier than the α1 subunits and at higher levels than the β or γ subunits. We also found that those cells that expressed α2δ1 would differentiate into muscle cells. This evidence indicates that the α2δ1 may be used as a marker of satellite cells that will differentiate into muscle.

1. Introduction

Satellite cells are found between the plasma membrane of the muscle fiber and the basal lamina [1]. They are responsible for the growth, maintenance, and repair of skeletal muscle. They remain in a mitotically quiescent state under normal physiological conditions but can be activated during exercise or muscle damage, aiding with the repair of muscle. Satellite cells can maintain or repair muscle because they possess stem cell properties; they can differentiate into other cell types [2] and can also divide and maintain their population. Activated satellite cells undergo several rounds of cell division, and some of them will differentiate and fuse to form the typical multinucleated skeletal muscle fiber. Due to the regenerative capacities provided by satellite cells, they are a valuable option for cell therapy. Cells used for therapy are selected based on specific markers present in the plasma membrane. In the case of satellite cells the function of those markers ranges from the regulation of proliferation to cell-cycle entry to fusion (reviewed in [3]). Although many markers have been identified, there is a need to find a marker indicative of the cells that will commit to the muscle lineage and that is expressed at different states, that is, activated, quiescent, or differentiated cell. Furthermore, the marker has to be present in human tissue. Here we examined the α2δ1 protein, a subunit of calcium channels that fulfills those requirements.

The α2δ1 subunit forms a part of the L-type voltage-dependent calcium channel (or dihydropyridine receptor, DHPR) in adult skeletal muscle. In addition to α2δ1, the skeletal muscle DHPR contains α1 (Ca,v1.1), β, and γ subunits. The α1 subunit is the voltage sensor and contains the channel pore [4], while the role commonly assigned to the other subunits is to regulate the activity of α1. However, recent evidence has shown that the β and the α2δ1 subunits have roles independent of calcium channels. The β subunit is an intracellular protein involved in the regulation of gene expression in different cell types including myoblasts [5–7]. The α2δ1 protein is an extracellular protein involved in cell attachment and migration and possibly cell signaling in myoblasts [8, 9]. We recently found that the α2δ1 subunit
localizes at the leading ends of myotubes with little or no association with α1 subunits 2 days after the induction of differentiation [8] suggesting that, in addition to attachment and migration, α2δ1 may play a role in the elongation process of myotubes. With longer times in differentiation medium, the localization of α2δ1 gradually becomes homogeneous until it colocalizes almost completely with α1. However, some α2δ1 subunit does not colocalize with α1 even at later times in a number of myotubes. Interestingly, experiments performed in dysgenic muscle (which lack the α1 subunit) have shown that the α2δ1 subunit is normally expressed but that its distribution patterns are abnormal in the absence of α1 [10]. In dysgenic muscle cells, α2δ1 was found in the plasma membrane, around the nucleus, and in the transverse tubular membrane in a diffuse pattern. Accordingly, the localization pattern of α2δ1 in dysgenic cells closely resembles our findings in immature muscle where there is little or no α1 subunit to associate with α2δ1. These data indicate that the α2δ1 subunit is not only part of the DHPR but that it may be important for other cellular functions in muscle precursor cells or satellite cells. Thus, the purpose of the present study was to determine whether the α2δ1 subunit is present in satellite cells and, if so, when the α2δ1 subunit first appears in those cells. We also sought to determine the fate of satellite cells expressing α2δ1. We found that the α2δ1 subunit is expressed in the majority of newly isolated satellite cells and that it appears earlier than the α1 subunits and at higher levels than the β or γ subunits. We also found that those cells that expressed α2δ1 would differentiate into muscle cells. This evidence indicates that the α2δ1 may be used as a marker of satellite cells that will differentiate into muscle.

2. Methods

2.1. Isolation of Satellite Cells. All experiments using animals were approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago. Skeletal muscle of newborn mice (<72 hours postnatal) was dissociated for fluorescence-activated cell sorting (FACS) and used for the extraction of total RNA (see below). Dissociation of muscle was performed in Ca2+-, Mg2+-free Rodent Ringer (in mM): 155 NaCl, 5 KCl, 11 glucose, 10 HEPES, pH 7.4 containing, 0.3% trypsin type XI, 0.01% DNAse I, and 1 mg/mL collagenase type IA (Sigma), as previously reported [11].

2.2. Fluorescence-Activated Cell Sorting. Cells were suspended in sorting media (phosphate-buffered saline (PBS) with 10 mM HEPES and 0.5% BSA) and labeled with antibodies against CD34 (anti-mouse CD34 conjugated with Alexa Fluor 700; eBiosciences) and α2δ1 (monoclonal antibody 20A from Pierce Thermo Scientific and a secondary pac blue goat anti-mouse antibody from Invitrogen). Collection tubes contained growth media (high-glucose DMEM supplemented with L-glutamine, 10% equine serum, 10% fetal bovine serum, and 1% penicillin/streptomycin). Immediately after the sort was completed, the cells were centrifuged, resuspended in Dulbecco’s modified essential medium supplemented with 4.5 g/L glucose, 10% horse serum, and 10% fetal calf serum, and plated at a density of 26,000 cells per square centimeter on 35 mm primaria dishes.

2.3. Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR). Total RNA was obtained from satellite cells from two 35 mm culture plates for each day (0–7). RNA extractions were done following Qiagen RNeasy mini-kit followed by DNase treatment to avoid amplification of genomic DNA. The RNA density for each sample was measured (Thermo Scientific: spectrophotometer NanoDrop 8000) and normalized to the lowest RNA density found in the sample group and reverse transcribed to cDNA using ImProm-II kit from Promega with a random primer. RT-qPCR was performed (Applied Biosystems 7500) using 10 μL of Fast SYBR Green Master Mix, 7 μL of molecular grade water, 1 μL of forward, and 1 μL of reverse gene specific primer, plus 1 μL of cDNA. All genes except 18S were run between 59 and 60°C annealing temperature, and dissociation curves were obtained for all gene/cDNA mixes and for the primer without cDNA for control and comparison. The 18S gene was run at 55°C. The number of independent cell cultures analyzed was a minimum of 3, and the same gene/cDNA mix was analyzed 3 times in qPCR 96 well plates. All genes were referenced to the geometric mean of at least two control genes selected from YWHAZ, 18S, and HPRT1 [12, 13]. The primer sequences are given in Table 1.

2.4. Statistics Data. Are expressed as means ± SEM. To determine statistical significance, we used one-way ANOVA followed by Tukey’s multiple comparison test or two-way ANOVA followed by Dunn’s multiple comparison test. A value of P < 0.05 was considered to be significant.

3. Results

3.1. Presence of α2δ1 Protein in Isolated Satellite Cells. Satellite cells were isolated from hind limb muscles from newborn mice and separated by FACS for the presence of the accepted marker CD34 [14]. CD34+ and CD34− cells were plated separately on primaria culture dishes and allowed to grow. CD34− cells divided, fused, and developed into myotubes, while most of the CD34+ cells remained mononucleated after 7 days in culture, as shown in Figure 1. Differentiation of CD34− cells into myotubes is consistent with other current studies showing that CD34 is lost after satellite cells are activated and that CD34+ cells experience little division and remain in a quiescent state [15]. We then performed a double-labeled sort and examined freshly isolated cells for the presence of α2δ1 since we had previously reported that this protein is expressed early in development of muscle cells and that it is involved in attachment and migration [8, 16]. We found that more than 50% of satellite cells expressed α2δ1 protein upon isolation, and the majority of these α2δ1+ cells were CD34+, as shown in Table 2. The four groups were plated separately in growth media (DMEM, 10% horse serum, 10% fetal bovine serum) and examined at different times in culture. Cells expressing α2δ1+ (CD34−)
Table 1: Sequences of primers used in qPCR measurements.

| Subunit    | Forward                                | Reverse                                |
|------------|----------------------------------------|----------------------------------------|
| α2δ1       | AGGCAGTTGAGATGGAGGAA                   | CCCCCCTTCTCCACCATTA                    |
| α1s        | AGGTGACGTGGCATGGACACTTAGA              | CCAAGTGCTACGGATGCTCCTAGA              |
| β          | CCGGACCTCGACGCTTGCTC                   | GGAATGGCGTCGCTGAGA                     |
| γ          | GCGGCTGCTGACTCCACCC                    | GGAGCCGGCACGGCCAG                      |
| MyoD       | AGGCTCTGTGCTGGCGCCAC                   | TGGAGCTTGGCGAGCCAG                     |
| Myogenin   | AGTGAATGCAACTCCCACGCC                  | GAGGGGCTTGCTGCTTACCCAC                |
| 18S        | AATTGACGGAAGGGACCC                      | TGGCGACGCAGCCCGCCAC                   |
| HPRT1      | TAAGTTGAG.item1200                      | TGGCGACGCAGCCCGCCAC                   |
| YWHAZ      | AACAGCTTTCGATGGAACCC                    | TGGGATCCGATGTCACCTA-AAC               |

CD34<sup>-</sup> CD34<sup>+</sup>

Figure 1: Satellite cells sorted for CD34. CD34<sup>-</sup> (left) and CD34<sup>+</sup> (right) cells were plated in growth medium (20% serum). CD34<sup>-</sup> fused to form myotubes while CD34<sup>+</sup> remained mononucleated after 7 days in culture. Images are 623 μm x 623 μm.

Table 2: Distribution of sorted cells by groups. Mean ± sem, n = 11.

| α2δ1<sup>-</sup>/CD34<sup>-</sup> | α2δ1<sup>+</sup>/CD34<sup>-</sup> |
|-------------------------------|----------------------------------|
| 21 ± 3.7%                     | 32 ± 4.5%                        |
| α2δ1<sup>-</sup>/CD34<sup>+</sup> | α2δ1<sup>-</sup>/CD34<sup>+</sup> | 3.0 ± 1.2%                        |

produced myotubes early (2-3 days) and persisted for longer times in culture (>21 days) than the other three groups (Figure 2). Cells α2δ1<sup>-</sup>/CD34<sup>-</sup> produced myotubes and also fibroblast-like cells; these myotubes did not last as long in culture as the ones produced in the absence of CD34. Cells α2δ1<sup>-</sup>/CD34<sup>+</sup> produced mostly fibroblast-like cells. The α2δ1<sup>-</sup>/CD34<sup>-</sup> cells produced myotubes at later times (>5 days) and did not last more than a few days in culture. Cells that were α2δ1<sup>-</sup>/CD34<sup>-</sup> at the time of the sort were then examined for the presence of α2δ1 with RT-PCR. It was observed that α2δ1<sup>-</sup>/CD34<sup>-</sup> cells started expressing α2δ1 after plating, and that they expressed α2δ1 only during the time when the cells were differentiating into myotubes. After 7 days in culture the level of expression of α2δ1 in α2δ1<sup>-</sup>/CD34<sup>-</sup> cells was 1.82 ± 0.07 (normalized to 18S). In α2δ1<sup>-</sup>/CD34<sup>-</sup> and α2δ1<sup>+</sup>/CD34<sup>-</sup> the level of expression of α2δ1 was 0.81 ± 0.03 and 1.01 ± 0.05, respectively, (n = 6).

These data further support the idea that α2δ1 is linked to the differentiation of the cells.

3.2. The α2δ1 Protein is Expressed Earlier Than the Other Calcium Channel Subunits. We further characterized the temporal expression of α2δ1 in the total satellite cell population at different times after isolation and compared it with the expression of α1, β, and γ subunits by RT-qPCR. The message for the α2δ1 subunit was present in freshly isolated satellite cells (D0) while the message for the other subunits was very low. To provide an objective comparison among the levels of expression of the different calcium channel subunits, Figure 3(a) shows the ratios of expression of α1, β, and γ subunits in relation to the expression of α2δ1 at D0. All values were normalized to the expression of α2δ1 since this subunit had the highest level of expression. The ratios of expression were 0.26 ± 0.04%, 2.89 ± 0.38%, and 5.89 ± 0.34% for α1, β, and γ subunits, respectively. These values were significantly lower than those of α2δ1 at D0 (P < 0.001).

Because the levels of expression of the subunits were so different at D0, it was expected that the expression for each subunit would not increase proportionately with differentiation. For the α2δ1 subunit its levels were significantly higher (6-fold) by D5 compared to D0 and remained high...
by D6 (Figure 3(b)). In contrast, levels of α1 were barely detected at D0. Low levels of α1 were detected by D1 but increased significantly (~70-fold) by D4 and stayed around this level through D6. Similarly to α1, the levels of β subunit increased more than 10-fold by D4 compared to D0. The γ subunit levels increased with time to reach a maximum 147-fold increase at D4 compared to D0. Thus, the fold increase in the levels of α1, β, and γ were substantially higher than for α2δ1. Overall, the mRNA data demonstrate that α2δ1 is expressed earlier than α1 and at higher levels than β or γ subunits in satellite cells and are consistent with studies detecting different protein levels of α2δ1 and α1 subunits early in development [9, 17].

3.3. Quantification of Cell Size. In order to obtain an objective aspect of cell morphology and development of each of the four subpopulations of sorted cells, we measured cell dimensions at several days after initial plating. To facilitate the measurement of differences over time, cells were plated and maintained in the initial growth media. Wide-field images were recorded from several random places in culture dishes. The maximum width and length were measured with Image J software. The maximum width varied between 8 and 12 μm for all cell groups at days 2 and 4 after plating. After 7 days in culture, the width increased in the α2δ1+/CD34−, α2δ1−/CD34+, and α2δ1−/CD34− groups, with the increase being more significant in the latter (P < 0.001). The width showed a small decline in the α2δ1+/CD34+ group (Figure 4(a)). Since myoblasts align end to end to form the typical myotubes, we measured the length of the cells and estimated its relation to cell width. The aspect ratio: width, a measure of elongation, is shown in Figure 4(b) for the four subpopulations at 4 and 7 days. A ratio of 1 would represent a spherical cell. The ratio at day 2 was close to 1 in all groups and is not plotted in the graph. The populations expressing α2δ1 at the time of sorting had the largest aspect ratios of the four groups at day 4 (α2δ1+/CD34−, 18.1 ± 6.3; α2δ1+/CD34+, 11.7 ± 1.5; α2δ1−/CD34+, 4 ± 0.7; α2δ1−/CD34−, 6.4 ± 0.8) and the ratio was even larger for the α2δ1+/CD34− group at day 7 (α2δ1+/CD34−, 15.8 ± 2.9; α2δ1+/CD34+, 20.2 ± 3.4; α2δ1−/CD34+, 4.8 ± 0.3; α2δ1−/CD34−, 6.4 ± 0.8). These measurements are consistent with the idea that cells expressing α2δ1 form myotubes, as noted above. By day 7 few α2δ1−/CD34− cells had formed myotubes. Instead, cells of this population were compact and multinucleated in appearance. The results are also consistent with the appearance of α2δ1 at the leading edges of immature muscle cells [8].

3.4. Detection of Myogenic Regulatory Factors. To determine further the differentiation state of the sorted satellite cells and the relationship with α2δ1 expression, total RNA was isolated from each of the four groups of cells two days after plating in growth media to measure the levels of the myogenic regulatory factors MyoD and myogenin. The values were normalized to the expression of 18S (Figure 5(a)). The presence of the two regulatory factors was detected in the four groups although in different proportions. Cells expressing α2δ1 had significantly larger expression of myogenin than MyoD (α2δ1+/CD34−, MyoD 0.29 ± 0.02, myogenin 0.54 ± 0.07, P < 0.01; α2δ1+/CD34+, MyoD 0.16 ± 0.03, myogenin 0.49 ± 0.05, P < 0.01). The difference in MyoD expression between the α2δ1+ expressing cells was also significant (P < 0.05). Cells without expression of α2δ1 at the time of sorting had a larger amount of MyoD message than myogenin (α2δ1−/CD34−, MyoD 0.48 ± 0.04, myogenin 0.02 ± 0.01, P < 0.01; α2δ1−/CD34+, MyoD 0.81 ± 0.07, myogenin 0.44 ± 0.03, P < 0.01). However, as mentioned above, α2δ1−/CD34− started expressing α2δ1 after plating and thus the differentiation of these cells into muscle cells was delayed in comparison to the cells that expressed α2δ1 during sorting. Levels of MyoD were also significantly different between the α2δ1− cells (P < 0.001). In contrast, the levels of myogenin were significantly different only in the α2δ1−/CD34− population compared to the other three subgroups (P < 0.001). The presence of MyoD and low myogenin is indicative of a population of proliferating cells, while high myogenin and low MyoD, and suggests that these cells are in the process of differentiation [18]. The relationship between these two myogenic regulatory factors can be better appreciated by the ratio of myogenin to MyoD shown in Figure 5(b). Cells expressing α2δ1 had significantly larger ratios than α2δ1− cells suggesting that α2δ1+ cells are
Figure 3: Expression of calcium channel subunits in satellite cells by RT-qPCR. (a) The $\alpha_2\delta_1$ subunit is expressed at significantly higher levels than the $\alpha_1$, $\beta$, and $\gamma$ subunits in freshly isolated unsorted satellite cells. Expression of $\alpha_1$, $\beta$, and $\gamma$ subunits was normalized to $\alpha_2\delta_1$ levels for comparison. The asterisk indicates statistically significant difference with all other data points ($P < 0.001$). (b) Temporal expression of calcium channel subunits in satellite cells cultured for different times in 20% serum. For each subunit, the fold change was calculated with their respective level at D0. The $\alpha_2\delta_1$ subunit showed a smaller fold change with time in culture when compared to the other three subunits, followed by the $\beta$ subunit. The $\alpha_1$ and the $\gamma$ subunits showed a large fold change of expression. Bars represent mean ± sem of three independent cultures measured in triplicate in the qPCR plate. Differences between data points are represented by lines. The asterisk represents differences with all other bars except those ones with an asterisk. For all graphs, the $P$ value was <0.05. All genes were referenced to the geometric mean of at least two control genes selected from YWHAZ, 18S, and HPRT1.
committed to the muscle lineage. These results agree with the different capacity of the cells in each of the four groups to form myotubes as described above and further support the idea that the expression of the α2δ1 subunit is a good indicator of the fate of satellite cells.

4. Discussion
Satellite cells are naturally involved in the maintenance and repair of skeletal muscle. This function of satellite cells is of paramount significance in patients with muscular dystrophy.
In order to improve the repair of dystrophic muscle, it is necessary to supply muscle with an adequate number of satellite cells with an intact copy of the affected gene. Thus, it is evident that the population of satellite cells used for therapy needs to be enriched with a marker that is indicative of the commitment of the cells to become muscle. Here we have shown that satellite cells that express the α2δ1 protein are more likely to differentiate into muscle cells in vitro.

Satellite cells that expressed α2δ1 at the time of sorting were more likely to differentiate into muscle cells than the cells devoid of this protein. Some of the α2δ1−/CD34− cells, however, also differentiated into muscle cells but at a much later time than cells expressing α2δ1. The α2δ1+/CD34− cells expressed α2δ1 after they had been in culture for several days, effectively turning into α2δ1+/CD34− cells; the absence of CD34 alone did not seem to be enough for the cells to commit to the muscle lineage at early times. This result suggests that it may be optimal to induce the expression of α2δ1 prior to cell sorting in order to obtain a larger population of cells that will differentiate into skeletal muscle.

Commitment of cells to the muscle lineage in the presence of α2δ1 was also demonstrated by the expression of the myogenic regulatory factors MyoD and myogenin two days after plating. α2δ1-expressing cells at time of sorting expressed relatively higher levels of myogenin than MyoD. In contrast, the levels of MyoD were higher than those of myogenin in cells without α2δ1 at time of sorting. This suggests that cells expressing α2δ1 are in a more advanced state of commitment toward muscle than cells without α2δ1.

Further support for our proposing a role for the α2δ1 subunit as a marker of muscle commitment of satellite cells is provided by the fact that α2δ1 appears earlier than the α1, β, and γ subunits and its levels remain high through the differentiation process. This is the first paper that examines the expression of all the calcium channel subunit in the same cell type. These results also indicate that the assembly of the skeletal muscle DHPR as a complex occurs at later times in development than it was previously believed [19, 20] since not all the subunits are expressed initially and simultaneously.

An important property of cells expressing α2δ1 is that they exhibit improved adhesion and migration in vitro compared to cells without α2δ1, as we previously demonstrated [8]. Interestingly, this capability imparted to the cells by the α2δ1 is not limited to muscle cells and has been confirmed by another laboratory using bone cells and bone matrix (Thompson & Farach-Carson, personal communication). Involvement of α2δ1 in attachment and migration is a significant attribute for the selection of this protein as a marker of satellite cells since few satellite cells have been shown to migrate great distances from the site of injection in dystrophic muscle. Further experiments must be performed to test the ability of α2δ1-expressing satellite cells to attach and migrate away from the site of delivery in dystrophic muscle. We previously observed that α2δ1 is found on the leading edges of cells, and here we show that cells that exhibit early expression of α2δ1 elongate faster than cells without α2δ1. In addition to migration and attachment, α2δ1 may be actively involved in elongation of myotubes. An additional advantage of the α2δ1 protein is that it is also present in human cells. This fact makes α2δ1 an ideal candidate to use when isolating cells because many of the other markers present in mouse cells have not been characterized in human cells, and therefore their presence is unknown [3].

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References

[1] A. Mauro, “Satellite cell of skeletal muscle fibers,” The Journal of Biophysical and Biochemical Cytology, vol. 9, pp. 493–495, 1961.
[2] A. Asakura, M. Komaki, and M. A. Rudnicki, “Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation,” Differentiation, vol. 68, no. 4–5, pp. 245–253, 2001.
[3] S. Kuang and M. A. Rudnicki, “The emerging biology of satellite cells and their therapeutic potential,” Trends in Molecular Medicine, vol. 14, no. 2, pp. 82–91, 2008.
[4] T. Tanabe, K. G. Beam, J. A. Powell, and S. Numa, “Restoration of excitation-contraction coupling and slow calcium current in dysgenic muscle by dihydropyridine receptor complementary DNA,” Nature, vol. 328, pp. 313–318, 1988.
[5] H. Hibino, R. Pironkova, O. Onwumere et al., “Direct interaction with a nuclear protein and regulation of gene silencing by a variant of the Ca2+-channel β1 subunit,” Proceedings of the National Academy of Sciences of the United States of America, vol. 100, no. 1, pp. 307–312, 2003.
[6] Y. Zhang, Y. Yamada, M. Fan, S. D. Bangaru, B. Lin, and J. Yang, “The β subunit of voltage-gated Ca2+ channels interacts with and regulates the activity of a novel isoform of PAX6,” Journal of Biological Chemistry, vol. 285, no. 4, pp. 2527–2536, 2010.
[7] J. Taylor, T. Zhang, L. Messi et al., “The Cavβ1 subunit regulates gene expression in muscle progenitor cells,” Biophysical Journal, vol. 102, no. 3, article 365a, 2012.
[8] K. García, T. Nahbani, and J. García, “The calcium channel α2δ1 subunit is involved in extracellular signalling,” Journal of Physiology, vol. 586, no. 3, pp. 727–738, 2008.
[9] J. Garcia, “The calcium channel α2δ1 subunit interacts with ATP5b in the plasma membrane of developing muscle cells,” American Journal of Physiology, vol. 301, no. 1, pp. C44–C52, 2011.
[10] B. E. Flucher, J. L. Phillips, and J. A. Powell, “Dihydropyridine receptor α subunits in normal and dysgenic muscle in vitro: expression of α1 is required for proper targeting and distribution of α2,” Journal of Cell Biology, vol. 115, no. 5, pp. 1345–1356, 1991.
[11] K. J. Alden and J. García, “Dissociation of charge movement from calcium release and calcium current in skeletal myotubes by gabapentin,” American Journal of Physiology, vol. 283, no. 3, pp. C941–C949, 2002.
[12] J. Vandesompele, K. De Preter, F. Pattyn et al., “Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes,” *Genome Biology*, vol. 3, no. 7, 2002.

[13] L. Grajales, J. García, and L. D. Geenen, “Induction of cardiac myogenic lineage development differs between mesenchymal and satellite cells and is accelerated by bone morphogenetic protein-4,” *Journal of Molecular and Cellular Cardiology*, vol. 53, no. 3, pp. 382–391, 2012.

[14] J. R. Beauchamp, L. Heslop, D. S. W. Yu et al., “Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells,” *Journal of Cell Biology*, vol. 151, no. 6, pp. 1221–1233, 2000.

[15] N. Ieronimakis, G. Balasundaram, S. Rainey, K. Srirangam, Z. Yablonka-Reuveni, and M. Reyes, “Absence of CD34 on murine skeletal muscle satellite cells marks a reversible state of activation during acute injury,” *PLoS ONE*, vol. 5, no. 6, p. e10920, 2010.

[16] T. Nabhani, T. Shah, and J. García, “Skeletal muscle cells express different isoforms of the calcium channel α2δ subunit,” *Cell Biochemistry and Biophysics*, vol. 42, no. 1, pp. 13–20, 2005.

[17] M. E. Morton and S. C. Froehner, “The α1 and α2 polypeptides of the dihydropyridine-sensitive calcium channel differ in developmental expression and tissue distribution,” *Neuron*, vol. 2, no. 5, pp. 1499–1506, 1989.

[18] M. Kottlors and J. Kirschner, “Elevated satellite cell number in Duchenne muscular dystrophy,” *Cell and Tissue Research*, vol. 340, no. 3, pp. 541–548, 2010.

[19] B. E. Flucher and C. Franzini-Armstrong, “Formation of junctions involved in excitation-contraction coupling in skeletal and cardiac muscle,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 15, pp. 8101–8106, 1996.

[20] F. Protasi, C. Franzini-Armstrong, and B. E. Flucher, “Coordinated incorporation of skeletal muscle dihydropyridine receptors and ryanodine receptors in peripheral couplings of BC3H1 cells,” *Journal of Cell Biology*, vol. 137, no. 4, pp. 859–870, 1997.