• Sarcomerogenesis is the process by which optimal sarcomere length is maintained during periods of muscle growth and stretch.1,2
• Satellite cells (SCs) are muscle stem cells that have long been hypothesized to play a regulatory role in sarcomerogenesis.3
• Children with Cerebral Palsy (CP) have a reduced SC population compared to typically-developing children.4
• Sarcomerogenesis is also impaired in children with CP, who develop overstretched sarcomeres during periods of long bone growth.5
• This plays a role in muscle weakness and contracture formation.

To better understand the role of satellite cells in regulating sarcomerogenesis and contracture development, our objectives were to:
1) Test if reduced SC number impairs stretch-induced sarcomerogenesis
2) Evaluate histologic changes in SC-depleted muscle after chronic stretch
3) Determine if SCs are required for prevention of contracture development during periods of gradual muscle stretch

EXPERIMENT 1: Does reduced SC number impair stretch-induced sarcomerogenesis?
• Targeted depletion of SCs was performed with Pax7-CreERT2+; Rosa26-DTA+ mice.6
  • Treated with tamoxifen (n=9) to induce satellite-cell depletion
  • Treated with vehicle (n=10) and treated with tamoxifen to control for mouse strain

Right hindlimb casted for 2 weeks in dorsiflexion to induce soleus to adduct
SC knockdown assessed with flow cytometry using galectin-3 and quinacrine (Figure 1)

• Soleus muscle harvested for architectural and morphological evaluation

Sarcomere number calculated to assess sarcomerogenesis (Figure 2, Bottom)

EXPERIMENT 2: Does SC-depletion lead to extra-cellular matrix (ECM) changes in response to chronic stretch?
• Hematoxylin and eosin staining and laminin labeling used to evaluate muscle morphology and myofiber area in muscles harvested during Experiment 1 (Figure 3)

EXPERIMENT 3: Are SCs required for prevention of contractures during periods of muscle stretch?
• Right hindlimb of Pax7-CreERT2+; Rosa26-DTA+ mice (n=10) casted for four weeks in plantarflexion (n=10) to induce the soleus to substract sarcomeres

At two weeks, mice treated with tamoxifen (n=5) or vehicle (n=5) to induce satellite cell knockdown
• At four weeks, casts removed and mice allowed free cage mobilization for four weeks (Figure 4, Top)

Mobilization induces progressive muscle stretch on shortened soleus muscles
• Models the effects of long-bone growth on muscle stretch during development

Satellite cell number calculated to assess sarcomerogenesis (Figure 4, Right)
• Maximal dorsiflexion angles measured during the recovery period (Figure 4, Bottom)

RESULTS

Figure 1: SC quantification using quadriceps and gastrocnemii
• SCs identified as Pax7+/CD45-/CD31- (CD45 serves as internal control)
• Significant knockdown of SCs (65-75%) upon treatment with Tamoxifen

Figure 2: Stretch-induced sarcomerogenesis in SC-depleted muscle
• Significant increase in sarcomere number in all treatment groups

Figure 3: Muscle histology changes after chronic stretch in WT and SC-depleted muscle
• Hyper-proliferation of ECM (A) in SC-depleted muscle compared to vehicle-treated control, consistent with fibrosis
• Reduced myofiber area (B) in SC-depleted muscle compared to vehicle-treated control (263 µm2 vs. 1396 µm2, p<0.005)

Figure 4: SC-depleted muscle response to recovery from shortening
• Sarcomere number does not fully recover if SC population is reduced (-13% vs. -3 %, p<0.05).
• Maximal passive dorsiflexion angle is dramatically reduced in SC-depleted cohort, revealing contracture development (-16° vs. 31°, p<0.05)

CONCLUSIONS

• A reduced cohort of satellite cells is sufficient for chronic stretch-induced sarcomere addition.
• Despite normal stretch-induced sarcomerogenesis, SC-depleted muscles display pathologic changes seen in muscles of children with CP
  • Fibroic extracellular matrix
  • Reduced myofiber area
  • Recovery from a shortened position by addition of sarcomeres is hindered when SC number is reduced.
  • Models long-bone growth in children with CP
  • Indicates that a decreased SC number in CP patients may be responsible for contracture formation during development

REFERENCES

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