Controlled Heat Stress Promotes Myofibrillogenesis During Myogenesis

Qiongyu Guo, PhD; Devin Miller, MD; Howard Wang, MD; Joseph Lopez, MD; Denver Lough, MD; Anand Kumar, MD

Disclosure/Financial Support: Supported by The Johns Hopkins School of Medicine Research Grant. None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this manuscript.

INTRODUCTION: Hyperthermia therapy has recently emerged as a clinical modality used to finely tune heat stress inside the human body for various biomedical applications.[1] Nevertheless, little is known regarding the optimal timing or temperature of heat stress that is needed to achieve favorable results following hyperthermia therapy for muscle regeneration purposes.[2] The regeneration of skeletal muscle after injury is a highly complex and coordinated process that involves a multitude of cellular mechanisms.[3] The main objective of this study was to characterize the effects of hyperthermal therapy on the overall behavior of myoblasts during myogenic differentiation.

MATERIALS AND METHODS: A murine C2C12 myoblast cell line was used in this study due to its morphological similarity to the primary myoblasts and its purity without contamination from other cell types. Various cellular processes, including myogenesis, myofibrillogenesis, hypertrophy/atrophy, and mitochondrial biogenesis, were examined using systematic cellular, morphological, and pathway-focused high-throughput gene expression profiling analyses.

RESULTS: We found that C2C12 myoblasts exhibited distinctive time and temperature-dependence in biosynthesis and regulatory events during myogenic differentiation. Specifically, we for the first time observed that moderate hyperthermia at 39 °C favored the growth of sarcomere in myofibrils at the late stage of myogenesis, showing universal up-regulation of characteristic myofibril proteins. Characteristic myofibrillogenesis genes, including heavy polypeptide 1 myosin, heavy polypeptide 2 myosin, alpha 1 actin, nebulin and titin, were all significantly upregulated ($p<0.01$) after C2C12 cells differentiated at 39 °C over 5 days as compared to those at 37 °C. Furthermore, moderate hyperthermia enhanced myogenic differentiation, with nucleus densities per myotube showing 2.2-fold, 1.9-fold and 1.6-fold increases when C2C12 cells underwent myogenic differentiation at 39 °C over 24 hours, 48 hours and 72 hours, respectively, as compared to those differentiated at 37 °C. Yet, atrophy genes were sensitive even to moderate hyperthermia, indicating that strictly controlled heat stress is required to minimize the development of atrophy in myotubes. In addition, mitochondrial biogenesis was enhanced following thermal induction of myoblasts, suggesting a subsequent shift toward anabolic demand requirements for energy production.

CONCLUSION: This study provides novel insight to the impact of hyperthermal therapy on regenerative forms of muscle healing and may provide greater understanding on the utility of bioengineered hyperthermal techniques in clinical medicines for those suffered with critical musculoskeletal injuries.

REFERENCES:

1. Chatterjee DK, Diagaradjane P, Krishnan S. Nanoparticle-mediated hyperthermia in cancer therapy. Ther Deliv. 2011;2(8):1001-14.

2. Oishi Y, Roy RR, Ogata T, Ohira Y. Heat-Stress effects on the myosin heavy chain phenotype of rat soleus fibers during the early stages of regeneration. Muscle Nerve. 2015;52:1047-56.

3. Yusuf F, Brand-Saberi B. Myogenesis and muscle regeneration. Histochem Cell Biol. 2012;138(2):187-99.