CELLULAR AND MOLECULAR ASPECTS OF MUSCLE PLASTICITY

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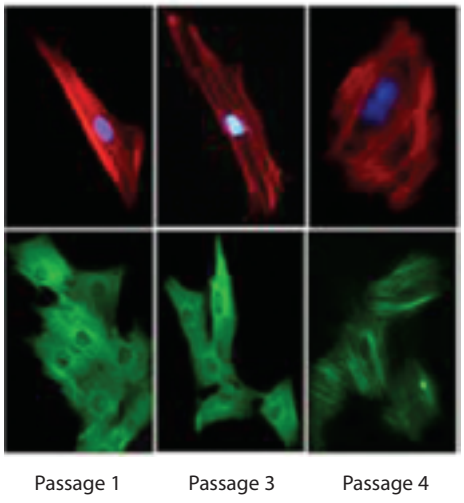


Figure 1. Representative immunostaining positive for SM α-actin (top) and calponin (bottom), magnification x200. Intense immunostaining for both markers was seen in passages 1-3. Note the elongation and assembly of actin filaments, and the spindle-shaped VSMCs from passage 1 through 3. Marker intensity decreased progressively from passage 4 on while VSMC morphology altered from spindle-shaped to polygonal, also seen at passage 5. At passage 10 VSMCs showed no marking for SM α-actin, which was seen only at the cell periphery, or for calponin, seen only at isolated points throughout the cytoplasm. Both markers decrease significantly in VSMCs submitted to freezing.

Muscles are tension generators that play a key role in 1) skeleton position, 2) moving blood along the circulatory system allowing tissue perfusion, 3) venous return, 4) peripheral vascular resistance control, 5) visceral movements and 6) ocular movements. Muscle tissues are highly plastic and respond quickly to injury and hormonal stimuli. Conditions in which muscle tissues are debilitated highlight their role in homeostatic control. For example, 1) a major cause of death in developed and developing countries is heart failure; 2) loss of skeletal muscle mass in severe cardiac failure is related to poor prognostic; 3) loss of skeletal muscle mass over aging is an important element of the senile syndrome. The development of new strategies aiming for a better outcome of muscle function necessarily relies upon a deeper knowledge of cellular and molecular biology of tissue responses. Therefore, the aim of this study is to gain further insight on cellular and molecular mechanisms underlying muscle plasticity. In subproject 1 the effect of certain mechanical stimuli will be stressed, to further investigate the role of Akt/mTOR on skeletal muscle mass control. In subproject 2, we will address the effects of increased skeletal muscle mass upon energy balance control. This will be achieved by using muscular IGF-1 transgenic mice. In subprojects 3 and 4 we will evaluate cellular and molecular effects of hormones extremely important to the homeostasis of muscle tissues: thyroid hormone (T3) and Angiotensin II. In subproject 5 a molecular approach of skeletal muscle proteolysis triggered by T3 will be performed. In subprojects 6 and 7, the activity of Akt/mTOR and calcineurin will be investigated in skeletal muscle of mice undergoing cardiac failure. Finally, in subproject 8, we will determine the effects of thyroid hormone and GC-24 (a thyroid hormone receptor , selective agonist) upon the global gene expression pattern in all three muscle tissues.

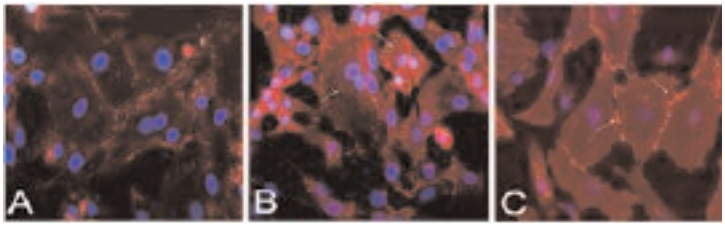
SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Subproject 4. VSMCs obtained from rat thoracic aortas by explant method were evaluated at passages 1 until 10 (fig. 1). From the 4th passage on, VSMCs underwent significant morphological changes from spindle- to polygonal-shaped and decreased differentiation markers. These data suggest that VSMCs until passage 3 may be employed as a model of differentiated phenotype but during later passages represent a model of dedifferentiated/proliferative phenotype. These findings highlight the importance of adequate manipulation of VSMCs since they undergo phenotypic modulation as a result of serial passages and freezing processes.

Subproject 6. By using KO mice which have sympathetic hyperactivity as a model for heart failure (HF), we showed that at 7 months of age HF mice displayed systolic dysfunction (32% vs. 24%, $p<0.05$) and exercise intolerance. Cross-sectional area of soleus and plantaris muscles was lower in 7 month-old HF mice with severe cardiomyopathy. Exercise training prevented soleus and plantaris muscles atrophy, since it significantly increased cross-sectional area in all fibers studied of both muscles. Analysis of reduced to oxidized glutathione ratio (GSH:GSSG) in WT and HF mice revealed that at 7 months HF mice displayed reduced GSH:GSSG ratio. Exercise training restored redox status of HF mice to age-matched WT mice levels. Taken together, these results suggest that exercise training by restoring skeletal muscle mass and redox state can be considered an important therapeutic strategy for preventing or reversing skeletal muscle myopathy in HF.

Subproject 8. Microarray analysis of cardiac tissue from rats submitted to a experimental hyperthyroidism revealed that out of the 30,000 sequences addressed, as many as ~8,000 are modulated 12hs, 24hs and 7 days after the onset of T3 treatment. Interestingly, about half of these genes were down-regulated. Genes associated with the extracellular matrix showed the highest Z score (percentage of genes regulated) in the array, reinforcing the broad impact of T3 in heart matrix remodeling. Aditional information in the array prompted us to investigate the effects of T3 upon B-catenin in the cardiomyocytes. T3 rapidly (30 min) increases B catenin protein levels in cardiomyocytes (fig. 2) but not in cardiac fibroblasts. In addition we found that pharmacological inhibition of PI3K severely decreases T3 dependent B catenin response. Considering that B catenin is a key regulator of cardiac hypertrophy, our results suggest that at least part of the hypertrophic effect of T3 might be mediated by B catenin throughout PI3K.

Figure 2 – Immunofluorescent staining against β-catenin (Red) and Cell Nuclei (DAPI-Blue), 200x. A- Control Cardiomyocytes, B-Cardiomyocytes treated with T3 for 30 minutes, C-Cardiomyocytes treated with T3 for 24 hours



MAIN PUBLICATIONS

Soares AG, Aoki MS, Miyabara EH, Deluca CV, Ono HY, Gomes MD, Moriscot AS. 2007. Ubiquitin-ligase and deubiquitinating gene expression in stretched rat skeletal muscle. *Muscle Nerve*. **36(5)**: Nov; 685-93.

Rolim NP, Medeiros A, Rosa KT, Mattos KC, Irigoyen MC, Krieger EM, Krieger JE, Negrão CE, Brum PC. 2007. Exercise training improves the net balance of cardiac Ca2+ handling protein expression in heart failure. *Physiol Genomics*. **29(3)**:246-52. Epub 2007 Jan 23.

Carrillo-Sepúlveda MA, Barreto-Chaves MLM. 2008. Effect of multiple serial passages and cryopreservation on phenotypic modulation in rat vascular smooth muscle cell cultures. *Histochemistry and Cell Biology*. Submitted.

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