Dr.ssa Polverino Valentina

Attività scientifica svolta nel 1° anno di Dottorato, Anno Accademico 2014/2015

Introduction

In skeletal muscle fibers, E-C coupling links the depolarization of the sarcolemma to Ca^{2+} release from the sarcoplasmic reticulum (SR), thus allowing muscle contraction. The muscle relaxation is marked with the refilling of calcium store; the SR depletion activates the plasma membrane store-operated calcium entry (SOCE). The cytosolic calcium is nfinally reuptaken into the SR by Ca^{2+}-ATPase (SERCA) and stored thanks to the major Ca^{2+} buffering protein Calsequestrin-1 (CASQ1). Recently, a point mutation in CASQ1 gene leading to the translation of a mutated (D244G) protein ($CASQ1^{D244G}$) affecting a high affinity Ca^{2+} binding-site, has been identified in patients with a mild myopathy. Accordingly, Ca^{2+} contraction in patient's skeletal fibers following caffeine stimulus, appear to be altered with respect to healthy controls. The aim of my project is to investigate whether $CASQ1^{D244G}$ altered CASQ1 ability to maintain elevated Ca^{2+} stores in the SR.

Methods

Plasmidic vectors carrying the coding sequence for wild type (wt) CASQ1-GFP or $CASQ1^{(D244G)}$-GFP were transfected in COS-7 and HeLa cells. Transfected cells were loaded with FURA2-AM, a fluorescent Ca^{2+} indicator. Cells were incubated in Ca^{2+} free buffer and treated with thapsigargin (TG), an inhibitor of SERCA pump. The amount of Ca^{2+} stored in the ER was indirectly estimated measuring elevation of $[Ca^{2+}]_{cyt}$ by fluorescence microscopy.

Results

Our results indicated that COS-7 cells transfected with $CASQ1^{wt}$ had a larger amount of stored Ca^{2+} with respect to $CASQ1^{D244G}$ transfected cells. We are now performing additional experiments on COS-7 cells transfected with $CASQ1^{wt}$ or $CASQ1^{D244G}$ without the GFP, in order to avoid interference in CASQ1 polymerization due to the steric hindrance of the tag-protein. In parallel, we evaluate the effect of $CASQ1^{D244G}$ on SOCE in transfected HeLa cells depleted of Ca^{2+} store by TG, after Ca^{2+} readmission. To analyse the unidirectional Ca^{2+} entry through SOCE we are also performing Mn^{2+} quenching assay.

Formative activities:

- "Corso di formazione sulla tutela della salute e sicurezza nei luoghi di lavoro. Lavoratori di area scientifica-alto rischio- 16 ore " (Universita di Siena) Siena, 26-27 Febbraio 2015.

- "Computational systems biology applied to pharmacology and nutrition" (Prof. Corrado Priami, Universita degli Studi di Trento) Siena, 2015.

- "Stress, inflammation and reproduction" (IBSA Foundation for scientific research, Universita degli Studi di Siena) Siena, 2015.

- "Start up an Technology Transfer" (Prof Lorenzo Zanni- Dott.Andrea Frosini), Siena 15 settembre 2015.

- "Sistemi di ricerca europei: Project Design e Gestione di progetti di ricerca" (Dott. Giancarlo Pichillo), Siena 16 settembre 2015.

Molecular Medicine PhD seminars:

- "Regulation of chemokine biology by atypical chemokine receptors" (Prof. Silvano Sozzani, Universita di Brescia) Siena, 2014.

- "Regolazione del processamento degli mRNA. il ruolo della proteina SAM68 nello sviluppo e nelle patologie umane" (Prof. Claudio Sette, Universita di Roma Tor Vergata) Siena, 2014.

- "Novel pathway for the targeting and integration of transmembrane proteins" (Prof. Nica Borgese, Universita di Milano) Siena, 2015.

- "MYCN and the MRN complex: how the replication stress response impacts on neural development and tumori genesis" (Prof. Giuseppe Giannini, Universita di Roma La Sapienza) Siena, 2015

- "Investigation of skeletal muscle Ca2+ homeoastasis in animals models" (Prof. Peter Szentesi, University of Debrecen, Hungary) Siena, 2015.