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Introduction

The sarcoplasmic reticulum (SR) is subdivided in junctional SR (jSR), where Ca^{2+} is released and longitudinal SR (lSR), specialized in the uptake of Ca^{2+}. jSR communicates with the t-tubules forming the triadic junction, where the ryanodine receptor interacts with several proteins forming the Calcium Release Units (CRU). The SR [Ca^{2+}] is ~20 mM and a large portion of this Ca^{2+} is bound to calsequestrin (CASQ), a Ca^{2+}-binding protein found within the SR lumen, buffering the free Ca^{2+} to ~1 mM and concentrating it near the CRUs. The CASQ molecule exists either as a monomer and polymers, depending on the Ca^{2+} environment: at low [Ca^{2+}] CASQ has a not folded structure; the increase of [Ca^{2+}] up to 1 mM leads to the formation of higher-order polymers capturing large amounts of Ca^{2+} in the back-to-back interface. A mutation in CASQ1 gene ($CASQ1^{D244G}$) has been identified in patients with a vacuolar myophaty characterized by the presence of aggregates.

Methods

To characterize the mutation we cloned the $CASQ1^{WT}$ and the $CASQ1^{D244G}$ in frame with GST that was subsequently removed by thrombin cleavage. Purified proteins were analysed by fluorescence spectroscopy to investigate conformational changes at increasing [KCl]. To determine whether the mutation could interfere with CASQ1 aggregation, turbidity measurements were performed by spectrophotometric analysis.

Results

$CASQ1^{D244G}$ showed higher fluorescence than the $CASQ1^{WT}$, suggesting structural differences between the two proteins. $CASQ1^{D244G}$ aggregated more rapidly than the $CASQ^{WT}$ in the presence of both 2 and 5 mM of $CaCl_2$. Turbidity measurements in presence of 0 to 5 mM $CaCl_2$ revealed that the $CASQ1^{D244G}$ had higher capability to form aggregates in a Ca^{2+}-dependent way with respect to $CASQ^{WT}$. The addition of EGTA confirmed that these results were due to the specific Ca^{2+} effect on proteins aggregation. Further experiments will be performed to investigate the polymerization of $CASQ1^{D244G}$ and $CASQ1^{WT}$ at physiological conditions.

Abstracts e partecipazione a congressi

- V. Del Re, S. Spinozzi, A. Gamberucci, V. Barone and V. Sorrentino. Characterization of a calsequestrin-1 mutation identified in patients affected by a vacuolar myophaty. Oral presentation. XII Annual Meeting I.I.M. 2015, 01-04 ottobre 2015.
- "Stress, inflammation and reproduction", IBSA Foundation for scientific research, 03 luglio 2015.

Partecipazione a corsi e seminari

- "Corso di formazione sulla tutela della salute e sicurezza nei luoghi di lavoro. Lavoratori di area scientifica –alto rischio- 16 ore", 12-13 febbraio 2015.
- Prof. Corrado Priami. "Computational systems biology applied to pharmacology and nutrition", 23 febbraio 2015.
- Prof. Silvano Sozzani. "Regulation of chemokine biology by atypical chemokine receptors", 21 novembre 2014.
- Prof. Claudio Sette. "Regolazione del processamento degli mRNA: il ruolo della proteina SAM68 nello sviluppo e nelle patologie umane", 1 dicembre 2014.
- Prof.ssa Nica Borgese. "Novel pathway for the targeting and integration of transmembrane proteins", 16 febbraio 2015.
- Prof. Giuseppe Giannini. "MYCN and the MRN complex: how the replication stress response impacts on neural development and tumorigenesis", 3 marzo 2015.
- Prof. Peter Szentesi. "Investigation of skeletal muscle Ca^{2+} homeostasis in animals models", 21 maggio 2015.

• Pubblicazioni scientifiche

- Virginia Barone, Davide Randazzo, Valeria Del Re, Vincenzo Sorrentino , Daniela Rossi. Organization of junctional sarcoplasmic reticulum proteins in skeletal muscle fibers. Journal of Muscle Research and Cell Motility, 2015.