Skeletal Muscle Disorders

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Functional effects of ryanodine receptor mutations linked to congenital muscle diseases

In skeletal muscle calcium is a key second messenger regulating contraction and the sarcoplasmic reticulum (SR) is the intracellular organelle involved in its regulation. The ryanodine receptor Ca2+ channel (RyR1) present on the terminal cisternae of the SR, is closely apposed to and is directly activated by, the dihydropyridine receptor an L-type Ca2+ channel functioning as voltage sensor. Upon depolarization, the voltage sensor undergoes a conformational change promoting the opening of the RyR1, leading to Ca2+ release from the SR; this elevation of the myoplasmic [Ca2+] is necessary for, and leads to, muscle contraction and this process is called excitation-contraction coupling (Fig. 1).

More than 700 RYR1 variants have been identified in patients worldwide, making this gene the primary target of neuromuscular disorders and accounting for over 30 % of mutations found in patients with congenital myopathies. Both dominant and recessive RYR1 mutations occur and usually associate with different phenotypes. Dominant mutations are causative of malignant hyperthermia/rhabdomyolysis/exertional heat intolerance and central core disease and functionally impact the channel's biophysical properties. Patients bearing recessive mutations are generally more severely affected, characteristically also display involvement of extraocular muscles and are diagnosed as having multi-minicore disease/centronuclear myopathy. The latter mutations have no major effect on the channel properties, but their presence is accompanied by profound biochemical changes in patients' muscles, including a significant reduction of the RyR1 protein content and high levels of expression of class II histone de-acetylases. The reduced RyR1 levels in the SR membrane cause a decrease of calcium release during excitation contraction coupling, leading to weak muscles.

Our laboratory focuses on determining the functional effect of RYR1 mutations with the long-term goal of developing a pharmacological strategy to improve muscle function in patients. To do so we use two main experimental models: patient-derived biological material and transgenic mouse models knocked in for mutations identified in patients. Muscle biopsies are evaluated biochemically and physiologically. Our results have demonstrated that muscle biopsies from patients carrying recessive RYR1 mutations show abnormally low RyR1 protein content, alteration of gene methylation and an increase in the content of chromatin modifying enzymes including class II histone de-acetylases and DNA methyl transferases. A mouse model we knocked in for a mis-sense mutation in one allele and a frameshift mutation in the other allele (DKI mouse, Fig. 2) exhibits severe muscle impairment, reduced levels of calcium release and disorganization of the muscle ultrastructure. Additionally, extraocular muscles from the transgenic mice have impaired excitation contraction coupling, as well as almost no EO-MyHC, the eye-muscle specific myosin heavy chain isoform. These results are consistent with the weak eye muscles of patients carrying recessive RYR1 mutations.

We are also interested in other aspects of skeletal muscle and in particular in identifying and validating the function of newly identified SR proteins. We have characterized a number of novel proteins including JP-45, junctate, SRP-27 and SRP-35; the latter is a membrane bound retinol-dehydrogenase converting retinol (Vitamin A) to all trans-retinaldehyde. Our results show that SRP-35 is involved in glucose metabolism, facilitating glucose uptake into skeletal muscle. We are currently investigating directly the role of Vitamin A in skeletal muscle physiology.

Taken together the results of our studies will have important implications especially since they will promote the development of pharmacological therapies to improve the quality of life of patients with disorders leading to a decreased levels of RyR1 and may shed new information regarding the link between metabolic disorders and skeletal muscle glucose metabolism.

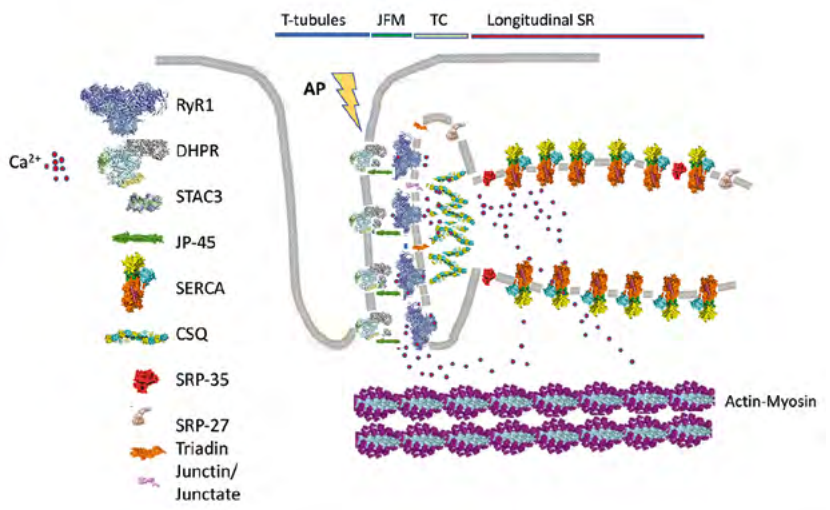

Fig. 1: Schematic representation of skeletal muscle proteins involved in excitation-contraction coupling. The figure shows the main components and subcellular localization of the proteins involved in skeletal muscle excitation–contraction coupling (ECC). The transverse (T-) tubules containing are invaginations of the plasma membrane where the voltage sensing dihydropyridine receptor (DHPR) is located. STCA3 binds to the DHPR. The T- tubules face the sarcoplasmic reticulum junctional0 face membrane (JFM) containing the ryanodine receptor 1 (RyR1) Ca^{2+} release channel, as well as JP-45, triadin and junctin/junctate/asparty β-hydroxylase. Calsequestrin bind Ca^{2+} and forms a mesh within the lumen of the sarcoplasmic reticulum. Opening of the RyR1 leads to Ca^{2+} release into the myoplasm which then binds to the contractile proteins resulting in sarcomeric shortening (muscle contraction). ECC is terminated when Ca^{2+} is actively pumped back into the sarcoplasmic reticulum by the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) Ca^{2+} pumps. SRP-35 is a retinol dehydrogenase converting retinol to *all* trans-retinaldehyde, SRP-27 (TRIC-A) oligomerizes on the terminal cisternae (TC) and longitudinal sarcoplasmic reticulum membrane where it is thought to be involved in the transport of K+ ions during Ca2+ release to maintain a neutral electrochemical gradient across the sarcoplasmic reticulum membrane.

Selected Publications

Eckhardt J, Bachmann C, Benucci S, Elbaz M, Ruiz A, Zorzato F and Treves S (2020). Molecular basis of impaired extraocular muscle function in a mouse model of congenital myopathy due to compound heterozygous Ryr1 mutations. Hum Mol. Genet. 29,1330–1339.

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Elbaz M, Ruiz A, Bachmann C, Eckhardt J, Pelczar P, Venturi E, Lindsay C, Wilson AD, Alhussni A, Humberstone T, *et al.* (2019). Quantitative RyR1 reduction and loss of calcium sensitivity of RyR1Q1970fsX16+A4329D cause cores and loss of muscle strength. Hum. Mol. Genet. 28, 2987–2999.

Bachmann C, Noreen F, Voermans NC, Schär PL, Vissing J, Fock JM, Bulk S, Kusters B, Moore SA, Beggs AH, *et al.* (2019). Aberrant regulation of epigenetic modifiers contributes to the pathogenesis in patients with selenoprotein N-related myopathies. Hum. Mutat. 40, 962-974

Ruiz A, Dror E., Handschin C, Furrer R, Perez-Schindler J, Bachmann C, Treves S and Zorzato F (2018). Over-expression of a retinol dehydrogenase (SRP35/DHRS7C) in skeletal muscle activates mTORC2, enhances glucose metabolism and muscle performance. Sci. Rep. 8, 636 doi: 10.1038/s41598-017-18844-3.

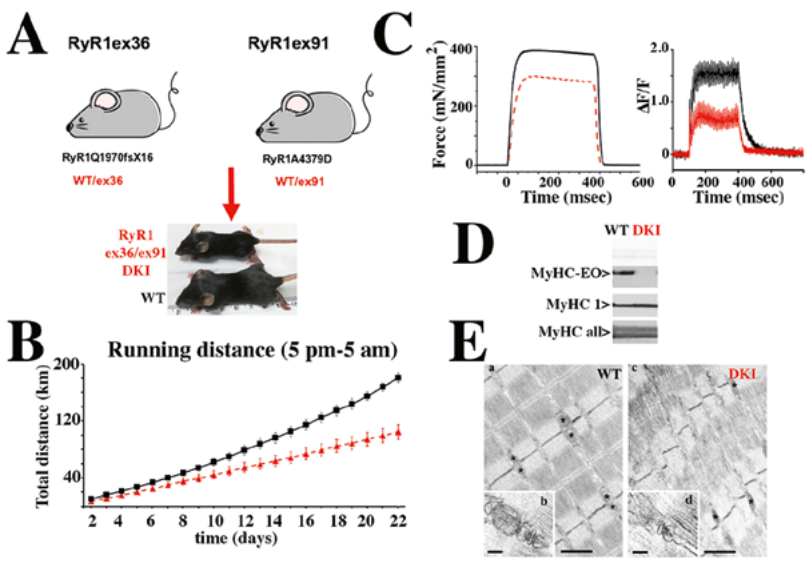

Fig. 2: Characterization of the DKI mouse model, knocked in for recessive RYR1 mutations. A. Double Knock In (DKI) mice were obtained by crossing heterozygous mice carrying a WT allele plus the frameshift mutation RyR1Q1970fsX16 with heterozygous mice carrying a WT allele plus the missense mutation RyR1A4329D. DKI mice (red) were on average 20% smaller than WT littermates (black). **B.** Muscle force assessed using the voluntary running wheel shows that DKI mice (red) run on average 50% less than their WT littermates (black). **C.** *Ex vivo* tetanic force stimulation of *externsor digitorum* longus muscles shows that muscles isolated from DKI mice (red) develop significantly less force than muscles from WT littermates (black)(left panel); the calcium transients elicited by electrical field stimulation at 150 Hz is also significantly reduced in EDL fibers from the DKI mice (right panel). **D.** EOMs from DKI mice show altered expression and content of the extra-ocular (EO) myosin heavy chain (MyHC) isoform. Top panel, membrane stained with Ponceau Red; central panel, blots stained with a monoclonal Ab specific for MyHC-EOM (top lanes) and a monoclonal Ab specific for MyHC1 (bottom lanes); bottom panel, blot stained with a monoclonal Ab recognizing all MyHC. **E.** Ultrastructure of EDL from WT and DKI mice. (a) In adult WT EDL fibers mitochondria are usually placed at the I band in proximity of Z lines (asterisks), next to CRUs. CRUs are mostly in the form of triads: two SR vesicles closely opposed to a central T-tubule (b). (c) In EDL fibers from DKI mice, mitochondria are less abundant and CRUs are often found in the form of dyads (d). Scale bars: a and c, 1 µm b and d, 0.1 µm.