
Skeletal muscle calcium homeostasis under normal and pathological conditions

Calcium is a universal second messenger regulating different biological functions from muscle contraction, to gene transcription and cell death. In skeletal muscle, Ca²⁺ regulates contraction and relaxation and alterations in its intracellular concentration can lead to neuromuscular disorders. Investigations carried out during the past decade have shown that in more than 50% of the cases, Central Core Disease, Multiminocore disease and Malignant Hyperthermia are linked to point mutations in the gene encoding the skeletal muscle sarcoplasmic reticulum calcium release channel ryanodine receptor (RyR1).

There are three isoforms of RyR that are expressed in different tissues; type 1 is preferentially expressed in skeletal muscles but recent data has shown that it is also expressed in some areas of the central nervous system, in some immune cells and in smooth muscle cells. These results imply that mutations in RyR1 may lead to alterations of Ca²⁺ homeostasis not only in skeletal muscle, but also in other tissues expressing this intracellular calcium release channel. Indeed ryanodopathies have recently been implicated in other clinical situations such as bleeding disorders, sepsis and intensive care polyneuropathy, broadening the clinical spectrum of disorders linked to altered RyR1 functions. Interestingly, type 3 RyR which was reported to be expressed at low levels in many tissues, appears to be the predominant isomorph in extraskeletal muscles and we are currently investigating its role in this group of muscles, by using a RyR3 KO animal model.

Our research also focuses on different pathways responsible for calcium regulation in skeletal muscle under normal and pathological conditions and on the identification of pathomechanisms in congenital muscular disorders. While most dominant RyR1 mutations affect Ca²⁺ homeostasis by changing the biophysical properties of the RyR1 Ca²⁺ channel, the mode of action of recessive RyR1 mutations is more elusive, especially since at the level of myofibers, effects on Ca²⁺ homeostasis are not prominent. On the other hand, striking changes occur in the patient’s muscles. Such changes include a drastic decrease of RyR1 protein content, depletion of muscle-specific mRNAs as well as depletion of mR32 and -34 specifically bound to the 3’UTR of the human RyR1, hyper-methylation of RyR1 CpG island and increased content of HDAC-4 and HDAC-5.

In order to gain insight into the mechanisms causing the epigenetic modifications and validate whether they represent valid pharmaceutical targets we have generated two mouse models harboring RyR1 mutations, using the CRISPR/Cas9 gene editing technology. The mutations we chose were originally identified in a severely affected patient with Multiminicore disease who harbored a premature stop codon in exon 36 (RyR1Gln19370X) and a missense mutation in exon 91 (RyR1 Ala432355Gp). Ex-II

Mutated RyR2 mRNA

HDAC 4.5

Methylation of CpG islands

Connection to Clinical Practice

Anesthesiology – a physiology lab?

Once feared as the most dangerous part of surgery, modern anesthesia has become very safe with an anesthesia-related mortality of below 1 in 200,000. Monitoring under general anesthesia includes heart rate, blood pressure, cardiac output, resistance, oxygen consumption, CO₂ production, minute ventilation, acid base status, neuromuscular function, urine output and much more. Today anesthesia has an impressive safety record. Nevertheless some diseases such as malignant hyperthermia (MH) are still life threatening. MH is a classical pharmacogenetic disease, triggered by anesthetic agents and characterized by a hypermetabolic state. Research has identified causative mutations in RYR1 in many susceptible individuals. However the genetic identity of some susceptible individuals is still unknown and needs to be determined in order to induce safe anesthesia. In addition neuromuscular blocking agents and volatile anesthetics can trigger severe skeletal muscle damage (myolysis, rhabdomyolysis) and hyperkalemia in patients with neuromuscular diseases. Many open questions on muscle physiology and calcium homeostasis are still to be understood in order identify patients at risk. Research on skeletal muscle excitation-contraction coupling and calcium homeostasis has the potential to further increase perioperative patient safety.

Fig. 1: Cartoon depicting how mutations in RyR1 lead to a decrease in RyR1 content thereby leading to weak muscles. Mutations lead to CHA hyper-methylation and HDAC4/HDAC5 over-expression. This causes mR32 sequestration thereby inhibiting transcription of genes regulated by mR32, including the RyR1 and muscle-specific mR35. A decrease in RyR1 would severely affect muscle excitation-contraction coupling since this calcium channel is a central player in this mechanism, releasing the calcium necessary for muscle contraction from the sarcoplasmic reticulum.

Fig. 2: Cellular distribution of RyR1 and Ca v1.1 in differentiated orbicularis oculi myocytes. Human myocytes were visualized with a Nikon A1 confocal microscope equipped with a C1p Apo TIRF 100X objective (1.49 N.A.) and stained as described in the Materials and Methods section. Top panels illustrate ocular, bottom panels EOM. Panels A and E anti-Ca v1.1 (green), B and F anti-RyR1 (red), C and G merged image of anti-RyR1, anti-Ca v1.1 and DAPI (blue); orange pixels show co-distribution of RyR1 and Ca v1.1. Panels D and H anti-Ca v1.2 (green). Bar indicates 20 μm

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