UNCONVENTIONAL MYOSIN VI AS A NOVEL REGULATOR OF MYOGENIC DIFFERENTIATION AND REGENERATION OF SKELETAL MUSCLES VIA MODULATION OF MITOCHONDRIA HOMEOSTASIS AND REDOX STATUS

DESCRIPTION FOR THE GENERAL PUBLIC

Skeletal muscles exhibit high plasticity and the capacity to adapt to various physiological demands. Regeneration of muscles is extremely important to maintain muscle functionality. Various steps of adult myogenesis during the process of regeneration were shown to be tightly regulated by a plethora of factors. Although a significant effort was put to understand the process of muscle regeneration, detailed mechanisms governing this phenomenon remain to be elucidated. Our recent results added new information about the role of one of the unconventional myosins in the function of skeletal muscles. The data obtained so far for myosin VI (MVI) provide evidence that this actin-based motor protein may play an important role in the differentiation of myogenic cells. We have shown that the mechanisms controlling cytoskeleton organization, as well as myoblast adhesion and fusion, were dysregulated in the absence of MVI. Consequently, this led to alterations in the process of myogenic cell differentiation. Lack of MVI resulted in the formation of aberrant myotubes, exhibiting spindle-like shape (myosac) morphology and centrally positioned nuclei. The abovementioned changes were accompanied by alterations in the redox status of myogenic cells reflected in a significant increase of the reactive oxygen species (ROS) levels and reduction in the activity of antioxidants. All these findings point to the alteration in redox homeostasis in MVI-knockout (MVI-KO) myogenic cells during differentiation. On top of that, we revealed the perturbation in redox state also in the hindlimb muscles isolated from MVI-KO newborn mice. The literature describes various steps of adult myogenesis and regeneration to be redox-regulated. It is well documented that high levels of free radicals can modulate signaling transduction and lead to pathological conditions in muscle tissue. Interestingly, we have observed a reduced percentage of intact mitochondria and reduced ATP levels in MVI-KO myogenic cells, pointing out the potential role of these organelles in ROS generation. Mitochondria aggregation is often associated with compromised mitochondria homeostasis and the development of oxidative stress. The same phenomenon was observed in MVI-KO myoblasts. It was accompanied by an elevated level of LC3 (autophagy marker) indicating an accumulation of autophagosomes in close proximity to impaired mitochondria. On top of that, a similar alteration of redox status was observed in the hindlimb muscles of newborn MVI-KO mice. Furthermore, in muscles of animals lacking MVI, activation of AMPK (5’-adenosine monophosphate-activated protein kinase, which level is upregulated by cellular stresses and ATP depletion) occurs, and protein translation is inhibited, indicating possible alterations in mitochondria status also in skeletal muscles. Thus, changes in the main metabolic pathways are remarkably reflected in the morphology of MVI-KO muscles: the cross-section area, as well as the number of nuclei per muscle fiber, is significantly reduced.

Taking into account, these findings as well as the crucial role of redox homeostasis, mitochondria status, and mitophagy (a selective form of autophagy) for myogenesis and muscle regeneration understanding molecular mechanisms of MVI involvement in the beforementioned processes may provide new information regarding this intriguing and not fully understood topic. To shed some light on these important aspects of MVI biology, in the current project we plan to identify the source of ROS generation and ROS type/types in MVI-KO myogenic cells and muscles. We are also interested in the investigation of MVI role in the maintenance of mitochondria homeostasis in the context of its involvement in mitophagy as a selective form of autophagy. The current project is also aimed to assess the molecular mechanisms of MVI involvement in signaling pathways orchestrating muscle regeneration and pathways associated with the redox status of skeletal muscle tissue. The work will be done on molecular, cellular, and tissue levels using different types of muscles isolated from Snell’s waltzer mice; these are natural MVI knockouts possessing spontaneous deletion in the corresponding gene. A set of widely accepted biochemical, molecular biology, genetic and electrophysiological techniques will be used in the proposed study. We expect that the results obtained in the frame of the proposal will provide not only new information about the role of MVI in muscle development and regeneration but also broaden our knowledge of the mechanisms related to muscle pathology associated with aberrations in myogenic differentiation and muscle regeneration.