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Aggregate-prone desmin mutations impair mitochondrial calcium uptake in primary myotubes

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ABSTRACT

Desmin, being a major intermediate filament of mature muscle cell, interacts with mitochondria within the cell and participates in mitochondria proper localization. The goal of the present study was to assess the effect of aggregate-prone and non-aggregate-prone desmin mutations on mitochondrial calcium uptake. Primary murine satellite cells were transduced with lentiviruses carrying desmin in wild type or mutant form, and were induced to differentiate into myotubes. Four mutations resulting in different degree of desmin aggregates formation were analyzed. Tail domain mutation Asp399Tyr has the mildest impact on desmin filament polymerization, rod domain mutation Ala357Pro causes formation of large aggregates composed of filamentous material, and Leu345Pro and Leu370Pro are considered to be the most severest in their impact on desmin polymerization and structure. For mitochondrial calcium measurement cells were loaded with rhod 2-AM. We found that aggregate-prone mutations significantly decreased $[Ca^{2+}]_{mit}$, whereas non-aggregate-prone mutations did not decrease $[Ca^{2+}]_{mit}$. Moreover aggregate-prone desmin mutations resulted in increased resting cytosolic $[Ca^{2+}]$. However this increase was not accompanied by any alterations in sarcoplasmic reticulum calcium release. We suggest that the observed decline in $[Ca^{2+}]_{mit}$ was due to desmin aggregate accumulation resulting in the loss of desmin mitochondria interactions.

1. Introduction

Desmin is a major intermediate filament protein (IF) of mature muscle cells. It has an organization typical for IF – helical rod domain flanked by nonhelical head and tail domains. Desmin filament assembly is a complex multi-step process and lateral annealing of rod domains is a key step in filament polymerization [1,2]. Consequently, desmin gene mutations residing in the rod domain result in severe disruption of the polymerization process, with intracellular aggregate formation and development of myopathies and cardiomyopathies [3–6]. Head and tail domain mutations often have less severe effects on filament structure but also lead to myopathies and cardiomyopathies by largely unknown mechanisms [7]. Some rare desmin single nucleotide polymorphisms (SNP) with only mild alterations in filament structure are also associated with development of muscle and cardiac disorders [8,9].

Desmin filaments are laterally connected to mitochondria [10], providing their proper positioning and architecture within the muscle cell under mechanical stress. Desmin knockout mice displayed abnormal mitochondrial morphology and spatial organization, significantly decreased oxygen consumption and increased ADP affinity [11]. Transgenic mice expressing desmin with the aggregate-prone Leu345Pro mutation demonstrated abnormal mitochondrial morphology, including reduced cristae density and vacuolization of mitochondrial matrix, and functional studies of these mitochondria revealed abnormal calcium uptake in comparison with wild type cells [12].

The ability of mitochondria to take up calcium from cytoplasm is provided by the mitochondria's close proximity to sarcoplasmic

Abbreviations: CmC, chloro-m-cresol; IF, intermediate filament; SNP, single nucleotide polymorphism; SR, sarcoplasmic reticulum; TMRE, tetramethylrhodamine, ethyl ester, perchlorate; WT, wild type mice.

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Table 1
Analyzed desmin mutations and their properties.

Aggregation capacity	Mutation	Desmin pattern in vitro	Phenotype
Aggregate-prone	Leu345Pro (DesL345P)	Formation of filamentous assembly precursors, disintegrated into small aggregates	Atrioventricular block, dilated cardiomyopathy [3]
	Ala357Pro (DesA357P)	Enhanced filament adhesiveness and aggregates formation	Skeletal myopathy, respiratory failure [23]
	Leu370Pro (DesL370P)	Formation of filamentous assembly precursors, disintegrated into small aggregates	Skeletal myopathy, respiratory failure [23]
Non-aggregate-prone	Asp399Tyr (DesD399Y)	Normal filaments formation	Atrioventricular block, dilated cardiomyopathy [25]

reticulum (SR) calcium releasing channels and thus exposure to calcium concentration several orders greater than bulk cytosolic calcium concentration. Under these conditions, the low affinity mitochondrial calcium uniporter is able to take up calcium from the cytosol resulting in increased calcium concentrations in mitochondria [13–20]. Calcium ions have a great impact on mitochondria functioning, since mitochondrial calcium uptake is involved in modulating ATP production in muscle cells [21,22]. Mitochondrial calcium overloading is widely believed to play a role in apoptosis and autophagy [15].

The aim of our study was to examine the effect of desmin gene point mutations of two types (aggregate-prone versus non-aggregate-prone mutations) on mitochondrial calcium uptake. Four mutations, all found in patients with myopathy/cardiomyopathy, resulting in different degree of desmin aggregates formation, were analyzed. The tail domain mutation Asp399Tyr has the mildest impact on desmin filament polymerization [6], the rod domain mutation Ala357Pro causes formation of large aggregates composed of filamentous material [23], while two other rod domain mutations, namely Leu345Pro [3,5] and Leu370Pro [23] are considered to have the severest impact on desmin polymerization and structure [6,24] (Table 1).

2. Materials and methods

2.1. Desmin cloning vector construction and mutagenesis

Murine desmin (*Des*, NM_010043.1) cDNA was cloned into a lentiviral plasmid vector pBK RSV. It was amplified via polymerase chain reaction to create restriction sites for AscI and SpeI on 5′- and 3′-ends. These sites were used for subsequent subcloning in a LVTHM plasmid. Site-directed mutagenesis was performed by means of polymerase chain reaction with primers containing the desired mutation. A set of plasmids carrying mutated genes of desmin was obtained and the following mutations were chosen for subsequent functional investigation: Leu345Pro (DesL345P), Ala357Pro (DesA357P), Leu370Pro (DesL370P), Asp399Tyr (DesD399Y).

2.2. Lentiviruses production

The LVTHM (20 μg), pMD2G (5 μg), and packaging pCMV-dR8.74psPAX2 (5 μg) plasmids were co-transfected into HEK-293T cells by a calcium phosphate method. The resultant production of lentivirus was concentrated by an ultracentrifugation method, resuspended in 1% BSA, frozen in aliquots at −80 °C, and titered using HEK-293T cells as described previously [25] (http://tronolab.epfl.ch/).

2.3. Satellite cells isolation and differentiation

Young (2–4 months) C57BL/6 male mice were supplied by B&K Universal (Sollentuna, Sweden). All studies were approved by Stockholm North Local Animal Ethics Committee and Local Ethics Committee of Federal Almazov Medical Research Centre. Mice were sacrificed by cervical dislocation. Muscles were removed and placed in DMEM with 1% penicillin/streptomycin (Gibco, USA). For satellite cells isolation, the soleus muscle was chosen since it contains a high percentage of slow fibres rich in mitochondria. Satellite cells were isolated as described elsewhere [26]. Muscles were digested in 0.1% collagenase I (Sigma, Germany) for 90 min at 37 °C. After digestion, cells were resuspended twice in DMEM supplemented with 10% horse serum and centrifuged at 1000 × *g* for 10 min. The pellet was resuspended in DMEM supplemented with 20% FCS, 10% horse serum and 1% chicken embryo extract. Cells were plated on Gel-trex (Gibco, USA) covered glass bottom Petri dish (Mattek, USA). Cells were cultivated until 80% confluence was reached. Next the cells were induced to differentiate by replacing the proliferation media with differentiation media (DMEM supplemented with 2% horse serum). Cell fusion was observed already after 12 h of cultivation in differentiation media. Satellite cells were left to differentiate for 7–10 days and the resulting myotubes were used for experiments.

2.4. Satellite cell transduction

For lentiviral transduction, concentrated viral particles were added to satellite cell suspension containing 8 μg/mL polybrene (Sigma, Germany), and incubated for 10 min ahead of plating.

The following day, cell monolayers were washed multiple times to remove residual viruses and fresh proliferation media was added. To assess the efficiency of transduction lentivirus encoding GFP was used.

2.5. Loading myotubes with calcium indicators

Calcium indicators were used to measure changes in free calcium ($[Ca^{2+}]$) within the cell. Rhod-2 AM (Molecular Probes, USA) was used to monitor Ca^{2+} in the mitochondrial matrix ($[Ca^{2+}]_{mit}$). Cytoplasmic calcium ($[Ca^{2+}]_i$) was monitored using either the ratiometric indicator, indo-1 AM (Molecular Probes, USA), or the non-ratiometric indicator, fluo-3 AM (Molecular Probes, USA). Cells were incubated for 30 min at room temperature with 5 μM rhod-2 AM, 2 μM fluo-3 AM or 5 μM indo-1 AM, then washed for 20 min with Tyrode buffer. Mitochondrial membrane potential was monitored with tetra-methyl rhodamine-ethyl ester (TMRE, Molecular Probes, USA).

2.6. Myotube stimulation for sarcoplasmic calcium release

Cells were stimulated chemically with 2 mM 2-chloro-m-cresol (CmC, Sigma, Germany) or continuously with 1 or 10 ms electrical individual pulses at various frequencies (1–100 Hz).