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Preaged remodeling of myofibrillar cytoarchitecture in skeletal muscle expressing R349P mutant desmin

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ABSTRACT

The majority of hereditary and acquired myopathies are clinically characterized by progressive muscle weakness. We hypothesized that ongoing derangement of skeletal muscle cytoarchitecture at the single fiber level may precede and be responsible for the progressive muscle weakness. Here, we analyzed the effects of aging in wild-type (wt) and heterozygous (het) and homozygous (hom) R349P desmin knock-in mice. The latter harbor the ortholog of the most frequently encountered human R350P desmin missense mutation. We quantitatively analyzed the subcellular cytoarchitecture of fast- and slow-twitch muscles from young, intermediate, and aged wt as well as desminopathy mice. We recorded multiphoton second harmonic generation and nuclear fluorescence signals in single muscle fibers to compare aging-related effects in all genotypes. The analysis of wt mice revealed that the myofibrillar cytoarchitecture remained stable with aging in fast-twitch muscles, whereas slow-twitch muscle fibers displayed structural derangements during aging. In contrast, the myofibrillar cytoarchitecture and nuclear density were severely compromised in fast- and slow-twitch muscle fibers of hom R349P desmin mice at all ages. Het mice only showed a clear degradation in their fiber structure in fast-twitch muscles from the adult to the presenescent age bin. Our study documents distinct signs of normal and R349P mutant desmin-related remodeling of the 3D myofibrillar architecture during aging, which provides a structural basis for the progressive muscle weakness.

1. Introduction

Muscle atrophy that occurs with aging (sarcopenia), disease and disuse are the prime factors for declining skeletal muscle performance, resulting in reduced mobility. Worldwide increasing life expectancies raise public interest in maintaining physical function in the elderly (Anton et al., 2015). Therefore, defining mechanisms triggering age-related muscle weakness is crucial to develop useful prevention and therapeutical approaches to address age-related diseases (Miller et al., 2014).

The age of symptomatic onset, clinical manifestation and progression rate in muscle diseases is extremely variable thus, patients may die young, suffer from progressing complications or display muscle weakness only at older age (de Rezende Pinto et al., 2015). An example of a group of progressive muscle disorders which aggravate during aging is the group of myofibrillar myopathies that are morphologically characterized by desmin-positive protein aggregates and myofibrillar degeneration (Schroder and Schoser, 2009). One classical protagonist out of the group of myofibrillar myopathies is the human desminopathy, which exists in autosomal-dominant and -recessive subforms. While the latter usually displays a childhood onset and a more severe cardiac and skeletal muscle phenotype, the autosomal-dominant forms are typically characterized by an adult onset between the third and the fourth decade of life (Clemen et al., 2013). Desmin, the major

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intermediate filament protein of muscle cells, serves to maintain myofibrillar cytoarchitecture and distributes externally applied mechanical stress intracellularly (Paulin and Li, 2004). Desmin and associated intermediate filaments form a 3-dimensional scaffold around Z-disks whilst linking myofibrils and mechanically and functionally connecting them to nuclei, mitochondria and sarcolemma (Schroder and Schoser, 2009). Over the last 2 decades, more than 70 disease-causing desmin mutations have been described; with the human R350P desmin mutation being the most frequently reported gene defect in desminopathies (Clemen et al., 2015). To study the sequential and molecular pathophysiology of desminopathies, we previously generated and characterized a R349P desmin knock-in mouse strain which harbors the ortholog to the human R350P mutation (Clemen et al., 2015; Winter et al., 2016). We exploited this desminopathy mouse model to study the effects of aging on the disease progression with special emphasis on structural alterations. Those might explain progressive muscle weakness due to hitherto unresolved structural remodeling of muscle architecture on the cellular and extracellular matrix level with age, as was described for instance in the scenario of muscular dystrophy (Friedrich et al., 2010).

Overall skeletal muscle contractility derives from the summed contribution of single muscle fibers, amongst other factors (Miller et al., 2014). Therefore, we analyzed the structural myofibrillar organization within single skeletal muscle fibers to test the hypothesis that progressive dysfunctions of muscle force in Des^{R349P} mice resulted from progressively deranged myofibrillar cytoarchitecture, as reported for mdx mice (Friedrich et al., 2010). We aimed to assess the whole aging aspect of progressive desmin-related myopathies. Thus, we present first quantitative data on age-dependent changes in sarcomeric architecture, nuclear density as well as desmin, myosin, and collagen content in fast-twitch extensor digitorum longus (EDL) and slow-twitch soleus (SOL) muscles from young, adult and aged Des^{R349P} mice applying state-of-the-art second harmonic generation (SHG) and quantitative morphometry. Our results clearly demonstrate progressive remodeling of muscle cytoarchitecture during aging in wild-type (wt), and exceedingly, in mutant Des^{R349P} mice to an extent that presents a preaged phenotype in the latter.

2. Materials and methods

Heterozygous (het) and homozygous (hom) littermates of the R349P desmin knock-in mouse model B6J.129Sv-*Des*$^{tm1.1Ccrs}$ (http://www.informatics.jax.org/allele/MGI:5708562) (Clemen et al., 2015; Winter et al., 2016) and wt littermate controls were used. Mice were divided into 3 age bins: 17–23 (young), 35–45 (adult) and 60–80 (aged) weeks of age. 60–80 weeks of age-old mice were chosen as oldest age bin, because survival of mice starts to decline at an age of >80 weeks (Chamberlain et al., 2007).

Sacrifice of animals and all experiments were approved by the local Animal Ethics Committee of the Friedrich-Alexander University Erlangen-Nuremberg and followed the guidelines of the Federation of European Laboratory Animal Science Associations. After sacrifice, SOL and EDL were dissected. Ringer's solution (RS) was exchanged to a Ca^{2+}-free "high K^{+}"-relaxing solution. The relaxed SOL and EDL were fixed using 1-fold Tris-buffered solution (TBS; 1060.1, Carl Roth GmbH, Karlsruhe, Germany) with 1% (v/v) non-acidic formaldehyde solution (P733.1, Carl Roth) for at least 72 hours at 4 °C. Single fibers were then manually tethered. For cryosections, EDL and SOL were dipped into liquid nitrogen-cooled isopentane (3926.2, Carl Roth) for 60 seconds, put into liquid nitrogen for 30 minutes, and stored at −80 °C. Cryosections of 10–30 μm thickness were prepared with a CryoStar NX 70 cryostat (Thermo Fisher Scientific, Waltham, MA, USA), collected on SuperFrost Plus adhesion slides and fixed with 1% formaldehyde solution-TBS. For vibratome sections, fixed SOL and EDL were embedded in 2% agarose (16500500, Thermo Fisher), stored at 4 °C and cut into 100 μm sections in a vibratome VT 1000S (Leica Biosystems, Nussloch, Germany). Total desmin was stained with mouse monoclonal antibody D33 (M0760, Dako, Hamburg, Germany) at 1:200 in 5% bovine serum albumin (A7030, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) -TBS overnight at 4 °C. Secondary antibody Alexa Fluor 594 (A11020, Molecular Probes Life Technologies, Darmstadt, Germany) was added 1:1000 in 5% bovine serum albumin-TBS for 1 hour. For nuclear staining, fixed single fibers were incubated in Ringer's solution supplemented with 10 μM Hoechst 33342 nucleic acid stain (PK-CA707-40047, PromoCell, Heidelberg, Germany) for 2 hours at room temperature (RT).

A multifocal multiphoton microscopy system (MPM; TriM Scope II, LaVision BioTec, Germany) with a mode-locked fs/ps-pulsed Ti:Sa-laser (Chameleon Vision II, Coherent, Santa Clara, CA, USA) was used to excite SHG signals of myosin, collagen, and multiphoton-excited fluorescence of respective stainings. Single fibers were z-scanned with a voxel-size of 0.139 × 0.139 × 0.500 μm^3 to detect verniers for calculation of vernier densities (VD, number of verniers (#) per fibers area of 100 μm^2), and to derive cosine angle sums (CAS). Compared images were taken under the same settings. For details see (Buttgereit et al., 2013; Diermeier et al., 2017; Friedrich et al., 2010; Garbe et al., 2012). The recorded z-stacks were displayed and analyzed with Fiji (National Institutes of Health, Bethesda, MD, USA). The collagen area was determined by subtracting autofluorescence from SHG signals, setting a threshold and calculating the area of the resulting collagen signal over the whole z-stack. Normalized to the myosin area, we obtained the area of collagen per area of myosin in μm^2/100 μm^2. Total desmin signals were analyzed by background subtraction, thresholding, and calculating the raw integrated density of signals normalized to the area of the whole z-stack. Autofluorescence signals of sections were determined by background subtraction, setting a threshold and the mean gray value of the whole z-stack. Nuclear density was analyzed using Imaris software (Bitplane AG, Zurich, Switzerland). Whole muscle homogenates for Western blots and myosin heavy chain isoforms (MHC) band separation were prepared by mechanical homogenization in 20 μL lysis buffer per mg of muscle. For sodium dodecyl sulfate polyacrylamide gel electrophoresis, 8% separation gels and 5% stacking gels with 100 μg of protein per lane were used. Gels ran at 150 Volts (RT) and wet Western blotting was conducted at 30 Volts (4 °C). Blots were blocked (5% milk-TBS for 1 hour at RT) and incubated with 1:500 anti-desmin (rabbit polyclonal, # ab8592, Abcam, Cambridge, UK) and 1:5000 anti-DesR349P [rabbit polyclonal antibody (Clemen et al., 2015)] overnight at 4 °C. After stripping (Stripping Buffer 46430, Thermo Fisher), blots were incubated with anti-glycerinaldehyde-3-phosphate-dehydrogenase (ABIN1107320, antibodies-online, Aachen, Germany) 1:5000 in 5% milk-TBS for 1 hour at RT. Horseradish peroxidase signals of the secondary antibodies (see Supplements) were detected by ECL substrate (1705061, Bio-Rad Laboratories, Munich, Germany), imaged with a Fusion-FX7-Spectra system (Vilber Lourmat SAS, Marne-la-Vallée, France) and analyzed with the Fiji gel analyzer tool. The intensities of the respective protein bands of total desmin and DesR349P were normalized to the intensities of corresponding glycerinaldehyde-3-phosphate-dehydrogenase bands. Separation of MHC in whole muscle homogenates of SOL followed (Roberts et al., 2012) with modifications. About 30% acrylamide (AA) with 50:1 acrylamide:bis and 10-fold electrophoresis buffer (0.5 M Tris, 0.75 M glycine, 17.34 mM SDS) were prepared. Separation gels contained 32% glycerol, 8% AA, 200 mM Tris (pH 8.8) 100 mM glycine, 0.4% SDS, 0.1% APS, and 0.05% TEMED (C. Lamboley et al., 2017, unpublished data). Stacking gels