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MINI-REVIEW

Biomechanical characterization of myofibrillar myopathies

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Myofibrillar myopathies (MFMs) are a group of sporadic and hereditary skeletal muscle diseases, which lead to severe physical disability and premature death. Most MFMs are caused by mutations in genes encoding desmin, plectin, VCP, filamin C, BAG3, FHL-1, αB-crystallin, DNAJB6, myotilin, and ZASP. Biomechanical studies on primary human myoblasts carrying desmin and plectin mutations showed increased stiffness and reduced mechanical stress tolerance i.e., higher mechanical vulnerability compared to control cells. Higher stiffness of mutant cells may lead to higher intracellular stress at physiologic stretch and shear deformation, which in turn could trigger muscle fiber degeneration.

Keywords: intermediate filament proteins; myoblasts; myotubes; mechano-transduction

Introduction

In degenerative CNS disorders including Parkinson, Alzheimer, Huntington, various forms of dementia and amyotrophic lateral sclerosis, morphological evidence of pathological protein aggregation has been established. Similar to this group of CNS disorders, pathological protein aggregation also plays an important role in a variety of human myopathies. Myofibrillar myopathies (MFMs) are a numerically significant group of progressive and devastating diseases of human skeletal muscle that often lead to severe disability and premature death. No causative or ameliorating therapy is yet available for this significant cohort of hereditary myopathies. MFMs are histopathologically characterized by desmin-positive protein aggregates, myofibrillar degeneration and mitochondrial dysfunctions. While about half of all MFMs are caused by mutations in genes encoding sarcomeric and extra-sarcomeric proteins (desmin, filamin C, plectin, VCP, FHL1, ZASP, myotilin, αB-crystallin, BAG3, and DNAJB6), the other half of these diseases are due to still unresolved gene defects (Schröder et al., 2007; Schröder and Schoser, 2009). The precise molecular pathways leading from an individual gene defect to a mutually shared myopathological disease manifestation remains to be determined (Ferrer and Olive, 2008).

Human desminopathies are the best-studied disease entity within the group of myofibrillar myopathies. Insights into the molecular pathogenesis of desmin mutations were obtained from transfection experiments and in vitro assembly studies, which indicate that the majority of desmin rod mutants are either (i) incapable of forming a de novo desmin intermediate filament (IF) network, (ii) forming abnormal IF structures, (iii) inducing the collapse of a pre-existing IF network, or (iv) leading to desmin-positive protein aggregates (Bär et al., 2005; Bär et al., 2006). These observations imply that mutated desmins compromise the filament formation competence and that filament-filament interactions are key events in the molecular pathogenesis. The question here still is, when these desmin mutants have such a toxic effect on the desmin filament system in vitro, why does it take so long until the clinical symptoms of progressive muscular damage become apparent in humans?

This previous "simple" mechanistic explanation was further challenged by observations that certain desmin mutants exhibit assembly defects in vitro when analyzed on their own, but facilitate proper filament formation when studied in one-to-one mixtures of mutant proteins with wildtype desmin. It is, however, unlikely that the complex human pathology is solely related to direct effects of desmin mutants on the assembly of desmin intermediate filaments. As an alternative explanation, desmin mutants may interfere with the interaction of binding partners, thereby influencing the structural, functional, and mechanical organization of

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the extrasarcomeric cytoskeleton as well as intracellular signaling cascades. This view was substantiated by the observation that mutations in genes encoding cytoskeletal linker proteins in muscle cells, namely plectin, filamin C, ZASP, FHL1 and myotilin, lead to a similar desmin-positive protein aggregation pathology. Several studies demonstrated that the desmin–plectin–filament system plays an essential role in the structural organization of the extrasarcomeric cytoskeleton by forming a three-dimensional scaffold around Z-discs, thus interlinking neighboring myofibrils and connecting the contractile apparatus to the sarcolemma, cytoplasmic organelles, and the nuclear membrane (Clemen et al., 2012; Winter and Wiche, 2013). Other contributing factors of MFMs relate to metabolic abnormalities such as mitochondrial alterations and to disturbances of protein quality control mechanisms (Schröder and Schoser, 2009) (Figure 1).

Biomechanics in myofibrillar myopathies

Most of the above-mentioned MFM proteins connect to adjacent myofibrils as well as to Z-lines ensuring proper anchorage in biomechanically active muscles. Disruptions of these linkages result in the disturbance of biomechanical properties that include elasticity, active force production, mechanical stress-induced cell damage, and insufficient muscle repair. Although the common symptom in all patients with MFMs is muscle weakness, there is almost no information at hand as to how muscle fibers are affected at different structural and functional levels within the organ, and as to the molecular causes of muscle weakness.

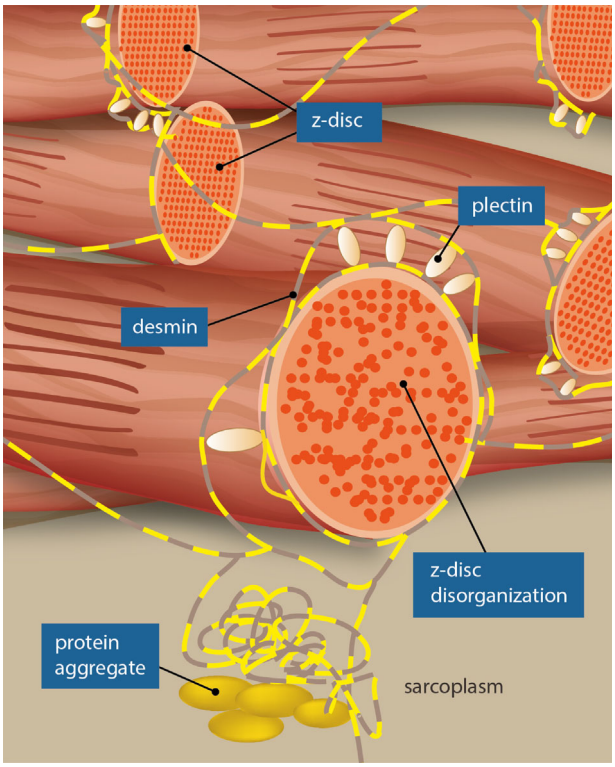

Figure 1 Schematic representation of skeletal muscle fibers that include the extramyofibrillar cytoskeleton. Mutations in proteins such as desmin and plectin can cause myofibrillar myopathies (MFMs), pathological protein aggregation, and Z-disc disorganization (Schröder and Schoser, 2009; adapted with permission).

Myoblast differentiation is a multistep process that involves the withdrawal from the cell cycle, acquisition of a cell type-specific transcriptional program and morphological changes that include elongation, alignment, and fusion of myoblasts into myotubes. While transcriptional regulation is at the core of myogenesis, the formation and growth of myotubes is controlled by a variety of signaling ligands, including FGF, Wnt, FAK, MAPK etc. The group of Adam Engler (Engler et al., 2004) investigated the influence of substrate stiffness on myoblast differentiation. They observed that upon myoblast anchorage to the substrate, the cells spread, withdraw from cell cycle, and fuse into nascent myotubes. Especially, the last step was sensitive to substrate compliance, pointing to an optimal, tissue-like matrix that is stiff and yet compliant enough to balance cell adhesion, contractility, and ultimately differentiation.

Myoblasts and myotubes offer in an in vitro system the advantage of representing a precursor cell system of muscle where cytoskeleton-membrane adhesions can be mostly studied in isolation without interfering effects arising from contractile filaments in fully developed muscle fibers. Using human myoblasts carrying a heterozygous R350P desmin mutation, we recently showed that pathogenic desmin mutations caused increased cell stiffness due to higher baseline contractile activation, leading to higher intracellular stress during cyclic stretch and consequently to a higher stress vulnerability in muscle (Bonakdar et al., 2012). In particular, desmin mutations and pathogenic alterations of the desmin-cytoskeleton led to an anisotropic and inhomogeneous intracellular stress distribution, and thereby to focal stress hotspots during stretch (unpublished observations). These hotspots have the potential to produce aberrant mechano-chemical signal transduction processes resulting in muscle cell damage. Problematic in these studies, however, was the large variability of patient-derived primary myoblasts, both from normal and diseased patients. Therefore, the use of better standardized cell lines was deemed necessary.

As a next step, we tested whether the biomechanical properties and the intrinsic mechanical stress response change in immortalized plectin-deficient mouse myoblasts. The most common disease caused by plectin deficiency, epidermolysis bullosa with muscular dystrophy (EB)-MD, is characterized by muscular dystrophy and severe skin blistering (Wiche, 1998; Wiche and Winter, 2011; Winter and Wiche, 2013). Compared to wildtype controls,

myoblasts lacking plectin revealed lower cell death (i.e., mechanical vulnerability) at 20–30% stretch and reduced substrate detachment in response to cyclic stretch on flexible membranes. Moreover, magnetic tweezer microrheometry using fibronectin-coated beads as well as traction force microscopy showed twofold higher stiffness and contractile forces in wildtype myoblasts compared to plectin-deficient cells. These findings provide first evidence of altered mechanical properties in plectin-deficient myoblasts and at early muscle development (Bonakdar et al., 2014; in press). Moving on from myoblasts to differentiated myotubes, we recently demonstrated that 10-days-differentiated wildtype myotubes are less vulnerable to external stress using the cell stretcher than plectin-deficient myotubes. Treatment with the aggregate-removing chemical chaperone 4-PBA had an ameliorating effect on plectin-deficient myotubes by clearly improving their resilience against mechanical strain (Winter et al., 2014). These observations support the notion, that unravelling underlying biomechanical properties of myoblasts and myotubes might one day also be advantageous for the development of pharmacological therapies for MFMs.

More detailed work should therefore be conducted on myotubes and eventually on myofibers to evaluate cellular mechanical properties of various MFM types. Moreover, principles like the corollary hypothesis that excessive mechanical stress causes aberrant mechano-chemical signal transduction processes resulting in muscle damage should be addressed for MFMs in future experiments..

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