Autophagy 9:3, 422–423; March 2013; © 2013 Landes Bioscience

Impairment of protein degradation in myofibrillar myopathy caused by FLNC/filamin C mutations

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Myofibrillar myopathy caused by FLNC/filamin C mutations is characterized by disintegration of myofibrils and a massive formation of protein aggregates within skeletal muscle fibers. We performed immunofluorescence studies in skeletal muscle sections from filaminopathy patients to detect disturbances of protein quality control mechanisms. Our analyses revealed altered expression of chaperone proteins and components of proteasomal and autophagic degradation pathways in abnormal muscle fibers that harbor protein deposits but not in neighboring muscle fibers without pathological protein aggregation. These findings suggest a dysfunction of protein stabilizing and degrading mechanisms that leads to a pathological accumulation of protein aggregates in abnormal fibers. Accordingly, a pharmacological modulation of chaperone activity may be a promising therapeutic strategy to prevent protein aggregation and to reduce disease progression. Newly established filaminopathy cell culture models provide a suitable basis for testing such pharmacological approaches.

Keywords: filaminopathy, myofibrillar myopathy, autophagy, chaperone-associated selective autophagy, ubiquitin proteasome system, heat shock proteins, immunolocalization studies

Abbreviations: CASA, chaperone-assisted selective autophagy; Ig, immunoglobulin; MFM, myofibrillar myopathy; UPS, ubiquitin-proteasome system

Submitted: 11/06/2012

Revised: 11/15/2012

Accepted: 11/16/2012

http://dx.doi.org/10.4161/auto.22921

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Punctum to: Kley RA, Serdaroglu-Oflazer P, Leber Y, Odgerel Z, van der Ven PF, Olivé M, et al. Pathophysiology of protein aggregation and extended phenotyping in filaminopathy.Brain 2012; 135:2642–60; PMID:22961544; http://dx.doi.org/10.1093/brain/aws200

Mutations in *FLNC*, the gene encoding filamin C, can cause myofibrillar myopathy (MFM) histologically characterized by focal disintegration of myofibrils starting at actin-crosslinking structures, called Z-discs, and massive protein aggregation within skeletal muscle fibers. FLNC consists of an N-terminal actin-binding domain followed by a semi-flexible rod comprising 24 highly homologous immunoglobulin (Ig)-like domains. Filamin dimers cross-link actin filaments in the Z-disc region and bind several Z-disc-associated proteins including MYOT, SYNPO2 and XIRP1. Biophysical and biochemical studies indicated that pathogenic mutations in *FLNC* leading to MFM cause protein misfolding and instability that triggers aggregation of mutant FLNC and subsequently many other proteins. Additional clinical and histological data revealed that muscle fibers can compensate for this effect for long periods of time. The pathomechanisms contributing to the manifestation of protein aggregation at an advanced age, however, are unknown. An impairment of protein degradation plays a decisive pathogenic role in various protein aggregate diseases, and we speculated that this is also the case for filaminopathy associated with an MFM phenotype.

We performed extensive immunolocalization studies on serial sections of skeletal muscle samples from MFM patients with *FLNC* mutations in order to monitor an impairment of protein quality control mechanisms. We analyzed the expression and distribution pattern of heat shock proteins, of key components of the ubiquitin-proteasome system (UPS) and of autophagic-lysosomal degradation pathways, including BAG3-mediated chaperone-assisted selective autophagy (CASA). Double immunofluorescence staining using antibodies directed against known aggregate proteins was performed

to detect abnormal fibers harboring protein aggregates.

A strongly increased immunoreactivity for the heat shock proteins HSPB6, HSPB1, DNAJB1 and HSPD1 is detected in areas of protein aggregation, whereas HSP90AA1 predominantly accumulates outside the aggregates in abnormal fibers. Markers of the UPS (UBB, FBXO32, PSMD4 and PSMB7) exhibit a distinct accumulation around and between aggregates but also, especially UBB, within protein deposits. In addition, we found a strong immunoreactivity for mutant UBB (UBB+1) in abnormal fibers. CASA components HSPB8, HSPA8 and SQSTM1 are markedly increased in aggregates, while the accumulation of STUB1 and BAG3 is more pronounced around the deposits. Immunoreactivity for autophagy markers MAP1LC3A and LAMP2 is also clearly enhanced in abnormal fibers. Reactivity for HDAC6 and VCP is particularly increased around and between aggregates, while a strong enhancement of TP53 is additionally detected in subsarcolemmal regions of abnormal fibers.

These findings suggest that abnormal muscle fibers react to protein aggregation with a strongly increased expression of proteins involved in proteasomal and autophagic degradation. However, there is also evidence for UPS and autophagy impairment. Accumulation of UBB+1 in abnormal muscle fibers points to UPS dysfunction, as observed in other protein aggregation diseases. A mechanism to compensate for UPS impairment caused by misfolded or aggregated proteins is upregulation of HDAC6-mediated autophagy. This may explain increased HDAC6 expression in muscle fibers harboring aggregates. Another compensatory response is induction of autophagy through a TP53-mediated pathway. This critically depends on the subcellular localization of TP53: Nuclear TP53 is capable of inducing autophagy through activation of *DRAM1* transcription and inhibition of the MTOR pathway, whereas cytoplasmic TP53 inhibits autophagy. The increased immunoreactivity observed for TP53 in the sarcoplasm of abnormal fibers therefore indicates an inhibition of autophagy rather than activation. The accumulation of SQSTM1 and MAP1LC3A in these muscle fibers further points to autophagy impairment. An age-related decline of proteasomal and autophagic activity may trigger aggregation of mutant FLNC and late disease onset. However, once aggregates accumulate, this may further impair the degradation systems and thereby aggravate the disease pathology.

Small heat shock proteins and other molecular chaperones facilitate proper protein folding and degradation of misfolded and aggregated proteins. A chaperone machinery essential for Z-disc maintenance (CASA), that consists of BAG3, HSPB8, HSPA8 and STUB1, facilitates the degradation of damaged Z-disc proteins like FLNC through an autophagic pathway. We observed increased expression levels of CASA components in abnormal muscle fibers but it seems that the general impairment of the cellular degradation systems limits the capacity of the CASA-mediating chaperones to dispose of damaged components and thereby prevent aggregate formation.

Intriguingly, the immunoreactivity for the examined components of the protein degradation machinery was not increased in muscle fibers without protein aggregates. This raises an essential question: Is a therapeutic induction of e.g., HSPs in early disease stages a possible strategy to prevent protein aggregation and to reduce disease progression? Initial studies in cell and mouse models of other MFM subtypes support this hypothesis. We established appropriate cell culture models of filaminopathy that show a massive aggregation of mutated FLNC resembling findings in patients' muscle fibers. This now enables us to analyze therapeutic approaches before testing them in animal models or patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.