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Summary

Titel: Molecular interactions of desmin and VCP in myofibrillar myopathies

Aim of this study was the investigation of changes of the proteasome- and autophagy-dependent protein degradation in two skeletal muscle diseases, the desminopathy and the inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia (IBMPFD). First, a luminescence-based proteasomal activity assay was established to determine the proteasomal activity. This assay is a highly sensitive, reliable and specific method which can be used with tiny amounts of skeletal muscle tissue. Results from proteasomal activity measurements showed that the specific proteasomal activity decreased with ageing and varied with individual muscle types.

A first attempt to generate a VCP R155C knock-in mouse line as physiological model for the human IBMPFD disease failed. Although PCR and Southern blot analyses indicated a correct targeting event, RT-PCR and Northern blot analyses showed no expression of mutant VCP mRNA. Importantly, our quantitative RT-PCR and Western blot analyses determined a decreased wild-type VCP expression in this VCP mouse model. From our data we conclude that we have generated a haploinsufficient VCP mouse line. Analyses of the proteasome and autophagy activities in these mice showed significantly reduced levels. This apparently resulted in a decreased protein degradation leading to the formation of tubular-like protein aggregates which we found in the skeletal muscle tissue of aged haploinsufficient VCP mice. The addition of purified VCP to skeletal muscle lysates increased the proteasomal activity. Since the human VCP shares 79% sequence identity with the *Dictyostelium discoideum* orthologue cdcD, a VCP R155C *D. discoideum* model was generated, too. This amoeba model will be used to more easily study aspects of the human IBMPFD disease, like the involvement of conserved signaling pathways in the pathogenesis of IBMPFD.

A desmin R349P knock-in mouse line as physiological model for the human desminopathy disease was successfully generated. Interbreeding of heterozygous animals resulted in homozygous animals, however, in lower numbers than expected. Western blot analysis of skeletal muscle protein extracts demonstrated a slower electrophoretic mobility of the R349P mutant in comparison to wild-type desmin. Analyses of desmin transcript and protein in heterozygous R349P mice showed an expression of approximately 40% mutant and 60% wild-type desmin. Two-dimensional gel electrophoresis revealed a shift to a more acidic pH in the case of R349P desmin. Together with the obtained biochemical data our first histopathological analyses revealed that the pathology in our R349P desmin mouse model mirrors the human desminopathy disease.