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Proteasome shuttling factors modulate the pattern of surface proteins of extracellular vesicles in a polyglutamine disease model

Authors:

Linda Kulka[1], Judith Riemer1, Diana Panfilova[1], Pia Fangmann[1], Gabriela Xavier[2,4], Alexander Navarrete Santos[3], Matthias Jung[2], Heidi Olzscha[1,5]

Affilations:

[1] Institute of Physiological Chemistry, Martin Luther University of Halle-Wittenberg, Germany
[2] University Clinic and Outpatient Clinic for Psychiatry, Psychotherapy, and Psychosomatic Medicine, Martin Luther University of Halle-Wittenberg, Germany
[3] Center for medical basic research, Martin Luther University of Halle-Wittenberg, Germany
[4] Morphology and Genetics Department, Universida de Federal de São Paulo, Brazil
[5] Institute of Molecular Medicine, Medical School Hamburg MSH, Hamburg, Germany

Abstract:

A characteristic pathophysiological sign of trinucleotide repeat disorders such as Huntington's disease (HD) is the aggregation of proteins with elongated repetitive stretches. It is not entirely clear, which components of the protein quality control (PQC) system contribute to the removal of misfolded proteins. Proteasome shuttling factors such as HR23A and HR23B play an important role in PQC, since they recognize ubiquitinated proteins and guide them to the proteasome.

We demonstrated that altered levels of the proteasome shuttling factors were able to influence aggregation of huntingtin (Htt) with a pathologically elongated polyglutamine (polyQ) stretch in human cell lines. Htt formed perinuclear aggregates, which differed in size and shape in HR23A and HR23B CRISPR/Cas9 knockout cell lines compared to the wild type. The proteins were detected via immunofluorescence microscopy after co-staining with proteostat, a dye intercalating with cross-beta structures.

One hypothesis claims that aggregated proteins can be exported by extracellular vesicles (EVs), which may contribute to their characteristic spreading pattern within the central nervous system. For this purpose, the content of EVs was analyzed by western blot and flow cytometry and Htt was found in the EVs. The surface proteins of EVs were determined by Magnetic Activated Cell Sorting (MACS) followed by flow cytometry in presence of different Htt constructs with or without ectopically expressed proteasome shuttling factors. We observed that HR23A and HR23B knockout in presence of polyQ proteins modulate the pattern of EVs surface markers. A variety of surface markers belonging to different pathways was increased including several markers involved in immune response. These effects could be partially reversed by HR23B overexpression.

Overall, the results demonstrate an effect of the proteasome shuttling factor levels on the aggregation of pathologically elongated Htt. It is possible that the levels of proteasome shuttling factors affect the spreading of the misfolded proteins mediated by EVs and may trigger a different immune response. Accumulation of aggregated Htt in cells can be reversed to some extent by overexpression of proteasome shuttling factors.