The role of the proteasome in Huntington's disease
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SHORT INTRODUCTION AND OUTLINE OF THE THESIS
INTRODUCTION

The cell can be considered as a big and crowded but well-functioning factory. Therefore, it is understandable that the proteome, which determines the status of the cell, needs to be monitored, controlled and adjusted very tightly. Proteostasis is thus an important aspect to retain homeostasis in the cell. The proteostasis network includes processes like protein folding and refolding by chaperones and protein degradation in order to prevent the accumulation and aggregation of hazardous misfolded proteins. Protein degradation is a continuous process, not only because of the limited half-life of many proteins and their natural turnover, but proteins can also become damaged by physiological conditions like stress and inadequate biogenesis [1, 2]. Moreover, aging, environmental and pathological conditions can induce increased damage or misfolding of proteins [3]. The latter can result in an overload of proteins targeted for degradation and consequently the misfolded proteins can sequester or interact with normal proteins causing loss of function.

Chaperones are one of the key players in the proteostasis network as they are involved in refolding proteins, prevention of misfolding and aggregation, and ultimately assist protein targeting towards the degradation machineries [4-6]. Upregulation of the amounts of chaperones can be a protective mechanism to prevent protein accumulation and aggregation during aging but also in various neurodegenerative diseases [7, 8]. Degradation of intracellular proteins occurs via two pathways, the ubiquitin-proteasome system (UPS) and autophagy. Autophagy is a lysosome-dependent degradation pathway which is generally held responsible for degradation of long-lived cytoplasmic proteins, large protein complexes and organelles [9]. The UPS mainly degrades short-lived and misfolded proteins. In this pathway, proteins are marked for degradation by a poly-ubiquitin chain that is recognized by the 19S cap of the proteasome [10]. This cap unfolds the protein, allowing access into the 20S proteolytic core where the protein is hydrolyzed into smaller peptides, ranging from 3 to 22 amino acids [11]. These peptides are subsequently processed into single amino acids by downstream peptidases or are presented by MHC class I molecules to the immune system.

While the UPS and autophagy are normally efficient in the turn-over of intracellular proteins, various neurodegenerative diseases, including Huntington's disease (HD), are hallmark by the accumulation of misfolded and aggregating proteins. HD is one of the nine polyglutamine (polyQ) disorders that are caused by an expansion of the polyQ tract in the disease-related protein, which is huntingtin in the case of HD, and causes misfolding of the protein [12-14]. Patients suffering from this fatal disease have late-onset movement disorders due to a slowly progressive degeneration of various neurological systems. HD remains elusive. The presence of proteasomes in polyQ aggregates in HD have led to the general idea that proteasomes are sequestered, leading to impairment of the UPS in HD [16]. In addition, proteasomes may be unable to degrade the polyQ tract, which would either lead to clogging of the proteasome by these polyQ fragments or the release of pure polyQ peptides [17, 18]. However, this data is conflicting with studies that monitored UPS activity in an HD mouse model, were aggregate formation did not necessarily lead to the accumulation of short-lived proteins [19]. This indicates that the UPS is functional in these models. In this thesis, the role of proteasomes in HD is studied to explore whether the proteasome can serve as a target for therapeutic intervention.

Outline of the thesis

With respect to the role of proteasomes in HD, many studies report contradictory results. In chapter 2 we discuss the background of HD and the UPS system, and review studies focused on proteasomes in HD and the hypotheses that were formulated in these studies. On the basis of the data presented, we hypothesize new strategies to improve mutant huntingtin (mHtt) degradation by modulating proteasomes. Because in vitro degradation studies of polyQ peptides suggests the inability of proteasomes to cleave within glutamines sequences, the toxic fragment hypothesis was formulated. This hypothesis states that the flanking sequences of mHtt are degraded by proteasomes but that pure polyQ peptides are released in the cell. In chapter 3, we designed polyQ constructs that mimic the polyQ peptides which may be released by the proteasome. We examined whether these peptides induce the same disease-related aggregation phenotype as polyQ-expanded proteins, indicating that polyQ peptides are initiators of aggregation, which may be a general mechanism behind all polyQ diseases. A comparative screen of multiple chaperones identified the chaperones DNAJB6 and DNAJB8 as suppressors of aggregation and toxicity induced by polyQ proteins. In chapter 4, we studied whether these chaperones prevent polyQ peptide aggregation. Additionally, we used chaperone mutants and FLIM microscopy to determine the mechanism of these chaperones in preventing polyQ aggregation. Since insufficient degradation of polyQ fragments was shown in in vitro experiments, using isolated proteasomes and short polyQ peptides, we examined in chapter 5 whether proteasomes can or cannot degrade polyQ-expanded mHtt fragments in living cells. We studied the role of proteasomes in mHtt degradation and generated short-lived mHtt variants that were directly targeted towards proteasomes in cells. In addition, we studied degradation of mHtt by proteasomes in vitro. In both cases we observed efficient degradation of the mHtt protein. To study the role of proteasomes in mHtt degradation in more detail, we explored and designed in chapter 6 various methods to study proteasomal functioning. We used fluorescent tags and activity probes to visualize proteasomal distribution, activity and interactions. These methods were subsequently used to study proteasomal behavior in mHtt-expressing cells. In chapter 7, various microscopical studies were used to explore proteasomal dynamics in polyQ-expressing cells. We analyzed whether proteasomes are indeed irreversibly sequestered into IBs, and whether this leads to proteasome inactivation. Because our studies indicate that proteasomes are still active in aggregates, that they are only reversibly recruited to aggregating mHtt and can still degrade mHtt, it raises the question whether proteasome activity can
be modified to improve mHtt degradation. In chapter 8 we studied the direct effects of proteasome modulation on mHtt degradation, both in vitro and in cellular systems.

REFERENCES