Mutant ubiquitin and the proteasome in Alzheimer's disease

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Neurodegenerative diseases in general are characterized by intracellular accumulation of aberrant proteins in specific brain areas. The ubiquitin proteasome system (UPS) is normally responsible for protein quality control and performs the majority of protein breakdown in the cell. A defective UPS is more and more implicated as a common factor in neurodegenerative diseases and its activity is diminished in Alzheimer’s disease brain. Affected neurons in Alzheimer’s disease generally accumulate UBB\textsuperscript{1}, a mutant ubiquitin carrying a nineteen amino acid C-terminal extension generated by a transcriptional dinucleotide deletion. Ubiquitin (Ub) is normally responsible for tagging proteins for degradation by the proteasome. Ub molecules form a branched tree of proteins by linking to substrates through isopeptide bonds between its C-terminal glycine residue and lysine residues in the substrate protein and in subsequent Ub proteins. This Ub tree represents a signal for protein breakdown by the proteasome. Chapter 1 is the general introduction of this thesis and presents a detailed view on the involvement of the UPS in Alzheimer’s disease.

In chapter 2, the properties of UBB\textsuperscript{1} are studied in a cell-free system and in human neuroblastoma cells. UBB\textsuperscript{1} does not participate in tagging proteins for proteasomal breakdown, since it lacks the C-terminal glycine residue that is necessary for this process. UBB\textsuperscript{1} is subject to ubiquitination itself, as the essential lysine residues are in the unchanged part of the protein. High expression of UBB\textsuperscript{1} resulted in apoptotic-like cell death.

Chapter 3 describes the specific inhibitory effect of UBB\textsuperscript{1} on the UPS, which therefore may contribute to proteasome inhibition as it is found in Alzheimer brain. In this study, a green fluorescent protein (GFP)-fusion-protein-based reporter system was used. The proteasome reporter system consists of fusion proteins of Ub and GFP with different linkers in between. The control fusion protein has a stable methionine residue in between, which results in a stable form of GFP after cleavage of the fusion protein by isopeptidases. The reporter construct does not contain a stable amino acid linker.
but has a glycine to valine mutation at the last amino acid of the Ub part of the fusion protein. Because of this mutation the reporter fusion protein is uncleavable to isopeptidases and is efficiently degraded as a whole by the proteasome. Therefore, cells expressing the latter construct will only accumulate GFP if proteasome activity is inhibited. In this system, UBB⁺¹ specifically and potently caused accumulation of the GFP reporter, indicating that UBB⁺¹ inhibits proteasome activity. Both proteasome inhibition and toxicity were found to be dependent on ubiquitination of UBB⁺¹ on two of its lysine residues. Paradoxically, this property also leads to UBB⁺¹ acting as a substrate for proteasomal degradation.

Chapter 4 discusses a probable explanation for this paradox, namely a threshold of UBB⁺¹ accumulation that needs to be surpassed to actively inhibit proteasome activity. This threshold hypothesis was studied in organotypical mouse cortex slice cultures and quantified in neuronal cell lines. Below the threshold, proteasomes can deal with UBB⁺¹, but if (through other mechanisms) proteasome activity is decreased, UBB⁺¹ accumulates and can contribute to further inhibition of proteasome activity. In Alzheimer's disease it seems that the threshold for UBB⁺¹ accumulation is exceeded, as the protein clearly accumulates in affected brain areas. However, the mutant UBB⁺¹ mRNA can also be found in control individuals and the protein is an efficient substrate for the proteasome at low concentrations. Therefore, it is not likely that UBB⁺¹ forms the initial trigger for proteasome inhibition in Alzheimer's disease, but more probable that other Alzheimer-related mechanisms cause this inhibition.

Chapter 5 deals with intracellular amyloid-β peptide (Aβ) formation as one of those mechanisms, which has been implicated in proteasome inhibition in Alzheimer's disease before. By expressing Ub-Aβ fusion constructs in cell lines, a purely cytosolic form of Aβ was induced. However, cytosolic Aβ was not able to inhibit proteasome activity in the GFP proteasome reporter system.

Finally, chapter 6 forms a general discussion of the results presented in this thesis and describes suggestions for future research. Additionally, preliminary data on tau aggregation and oxidative stress as proteasome-inhibiting factors in Alzheimer's disease are presented. From these data, no effect of soluble tau on proteasome activity was observed so far. Interestingly, oxidative stress was found to synergistically inhibit the proteasome with UBB⁺¹, but only under certain conditions that need further study.
In Alzheimer brain, initial proteasome inhibition can lead to accumulation of UBB*1 up to critical levels, which can subsequently form an important contribution to further inhibition of the UPS in and eventually to neurodegeneration. Additionally, UBB*1 can be regarded as an endogenous reporter of proteasome activity. Intriguingly, UBB*1 only seems to accumulate in tauopathies and not in synucleinopathies. More research on the mechanism of accumulation and proteasome inhibition by UBB*1, possibly synergistically with other processes, will provide more insight in discriminating between the different molecular backgrounds and pathogeneses of these diseases.