Protein Aggregation, the Ubiquitin-Proteasome System and Neurodegenerative Diseases

Protein aggregation is thought to be the pathological driving force responsible for neurodegenerative disorders such as Alzheimer’s, Parkinson’s, Huntington’s and prion diseases. However, it is not yet clear whether, or to what extent, the misfolded proteins are the cause of the disease rather than the consequence. The aggregated proteins that are characteristic of these diseases have in common the ability to undergo conformational changes and often form fibrillar structures (Figure 1). It is postulated that these proteins may share similar pathways of aggregation. Degradation of intracellular proteins via the ubiquitin-proteasome system (UPS) is a highly complex, temporally controlled and tightly regulated process that plays major roles in a variety of cellular processes and aberrations in this system have been implicated, either as a primary cause or secondary consequence, in the pathogenesis of neurodegenerative disease. This review will give a brief overview of the possible role of proteasome dysfunction and protein aggregation in neurodegeneration.

Prion diseases

Transmissible spongiform encephalopathies (TSEs) or prion disorders are rare fatal neurodegenerative disorders which include Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle and scrapie in sheep. A hallmark of these disorders is the accumulation of abnormal prion protein (PrPSc) which is a misfolded form of the normal cellular host prion protein (PrPC). The key pathological features of prion disorders are spongiform degeneration of the brain, massive neuronal loss, astrogliosis, and the accumulation in the CNS of PrPSc. However the precise cause of neurodegeneration in these disorders is not well understood, and a major gap exists in our understanding of how the conformational conversion of PrPC to PrPSc ultimately kills neurons.

Lindquist and colleagues have suggested a novel mechanism involving inhibition of the UPS and altered trafficking of PrPSc that may account for prion-associated toxicity. They propose that in neurons misfolded PrPSc is retrogradely transported to the cytoplasm via ER-associated degradation (ERAD). Whereas normally PrPSc reaching the cytosol would be rapidly degraded, they demonstrated using inhibition of the UPS with high-dose proteasome inhibitors in mouse neuroblastoma N2a cells that cytoplasmic accumulation of large quantities of PrPSc resulted in aggregates in the cytoplasm, which acquired properties of partial protease-resistance with self-sustaining properties of replication, thereby inducing neuronal cell death. However other reports argue against this potential neurotoxic species by suggesting that PrPSc does not undergo ERAD. Paradoxically one group found retro-translocated cytoplasmic PrPSc was actually neuroprotective in primary cultured neurons.

Table 1. Neurodegenerative diseases: aggregation in disease

<table>
<thead>
<tr>
<th>Disease</th>
<th>Protein deposit – major composition</th>
<th>Characteristic pathology</th>
</tr>
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<tbody>
<tr>
<td>Prion disease</td>
<td>Prion protein</td>
<td>Extracellular amyloid plaques, intracellular deposits, and occasional synaptosomal and axonal deposits</td>
</tr>
<tr>
<td>Huntington’s disease</td>
<td>Mutant Huntington</td>
<td>Intranuclear neuronal inclusions, cytoplasmic aggregates and fibrillar huntingtin segments</td>
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<tr>
<td>Parkinson’s disease</td>
<td>α-synuclein</td>
<td>Intracellular Lewy bodies, Lewy neurites, fibrillar α-synuclein</td>
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<tr>
<td>Alzheimer’s disease</td>
<td>β- or tau</td>
<td>Extracellular neuritic plaques, intraneuronal neurofibrillary tangles of hyperphosphorylated tau</td>
</tr>
<tr>
<td>Amyotrophic lateral sclerosis</td>
<td>SOD-1</td>
<td>Intraneuronal inclusions, insoluble SOD</td>
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</tbody>
</table>

Figure 1: Aggregation in neurodegenerative diseases. (A) Immunoreactive aggregates in neuronal nuclei (long arrows) and perikarya (short arrows) localised in Huntington’s disease cortex (with permission from Cotekust et al). (B) Hyperphosphorylated tau forms neurofibrillary tangles in neuronal cell bodies (Hippocampus of Alzheimer’s disease brain). (C) Halo immunostaining of Lewy bodies in Parkinson’s disease that predominantly comprises α-synuclein. The lighter granular material beside the Lewy body represents the neuramelin of dopaminergic neurons of the substantia nigra. (D) Extracellular plaque in sporadic prion disease stained with anti-PrP monoclonal antibody.

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human neurons by protecting against Bax-medi-
ated cell death. It seems likely that some propor-
tion of misfolded PrP\textsuperscript{\alpha} undergoes ERAD–proteasome-mediated degradation,\textsuperscript{b} but is likely to be rapidly degraded and a short-lived species; there is still clearly debate as to whether accumulation of this species is neurotoxic to the cell. It has been reported that proteasome dysfunction occurs in vivo in the brains of prion scrapie-infected mice; in this study they found that the level of ubiquitin–protein conjugates increased significantly ~144 days post infection when clinical signs first became apparent and that this elevation correlat-
ed with a decline in proteasome function.\textsuperscript{1} It is likely therefore that proteasome impairment may be important in the pathogenesis of prion dis-
ease, but may be unrelated to the accumulation of the wild-type prion protein, and work is ongoing in our laboratory to address this question.

**Huntington’s disease**

Huntington’s disease (HD) is an autosomal dom-
inant neurodegenerative disease with a prevla-
ence of symptom expression in Western European descent. In 1993, the gene defect associated with HD was identified as a CAG repeat expansion, encoding polyglutamine repeats, within a novel protein huntingtin. This highly polymorphic CAG repeat encoding polyglut-
amine is located in exon 1 and has been shown to range between 10 and 29 copies on normal chromosomes, whereas it is expanded to a range of 36–121 on HD chromosomes. HD is charac-
terised by selective loss of neurons in the striatum and cerebral cortex, and by nuclear and cytoplas-
mic inclusion bodies of aggregated fragments of N-terminal mutant huntingtin.\textsuperscript{1} It is not clear what role these aggregates play in the pathogene-
sis of HD. However, there is a striking correlation between the threshold for aggregation in vitro and the threshold for disease in humans, consist-
tent with the idea that aggregation is related to pathogenesis.\textsuperscript{1} This supports the argument that self-association or aggregation of expanded polyglutamine underlies a toxic gain of function. Bence and colleagues found that mutant hunt-
ingtin fragments inhibited proteasome function in intact cells, and the formation of intracellular aggregated inclusions was associated with a fur-
ther decline in UPS function resulting in a positive feedback mechanism that may explain the rapid loss of neuronal function in many neu-
rodegenerative diseases.\textsuperscript{10} Whether a decline in UPS dysfunction alone is sufficient to induce stri-
atal neuronal damage in HD was recently investi-
gated by Seo and colleagues, who found inhibi-
tion of the UPS in various brain regions from early and late-stage HD patients and also inter-
estingly in their skin fibroblasts.\textsuperscript{11} However, they report that UPS dysfunction is associated with neuronal pathology only when it occurs in paral-
lel with other neurodegenerative pathways involving decreases in brain derived neurotroph-
ic factor levels and defects in mitochondrial res-
piratory chain complex II, both of which are found in HD brains. A novel method of trying to ascertain whether HD inclusion bodies are path-
genic, incidental or a beneficial coping response has been reported recently by Arrasate and col-
leagues who developed an automated robotic microscope that returns to precisely the same neuron after arbitrary intervals.\textsuperscript{12} They found by studying neurons expressing mutant huntingtin fragments that inclusion bodies were not essen-
tial for neuronal death. They argue that inclusion bodies were in fact a by-product that lead to decreased levels of mutant huntingtin and thus improve survival, and suggest that the toxic species may, in fact, be an intermediate soluble form of the protein.

**Parkinson’s disease**

Parkinson’s disease (PD) is a common age-relat-
ed neurodegenerative disease that is characterised pathologically by extensive selective and progres-
sive neurodegeneration in the substantia nigra (SN) of the midbrain with resultant loss of dopamine in the striatum, and formation of Lewy bodies in the remaining neurons in the SN as well as in a few other brain regions.\textsuperscript{13} It is also clear that mitochondrial dysfunction and oxidative stress in sporadic PD, UPS impairment may also con-
tribute to SN dopaminergic pathology. Lewy bodies are cytoplasmic inclusion bodies of fibrillar,
misfolded proteins containing ubiquitinated \(\alpha\)-synuclein and parkin as the major constituents although proteasome components are often found. Sporadic PD has been shown to be asso-
ciated with impaired 26/20S proteasomal func-
tion and in 2002, McNaught and colleagues showed that inhibition of 26/20S proteasome function resulted in the specific accumulation of \(\alpha\)-synuclein and ubiquitin, with the formation of cytoplasmic inclusions that were immunoreac-
tive for these proteins, in dopaminergic neurons in culture.\textsuperscript{14} More recently, they showed that pro-
teasome inhibition alone is sufficient to produce a clinical syndrome in rats that closely recapitu-
lates key features of PD and not more widespread neuronal damage.\textsuperscript{15} Whether such inclusions contribute to neuronal death or protect cells from the toxic effects of misfolded proteins remains controversial.\textsuperscript{16} Genetic defects in UPS protein components have been found linked to familial forms of PD. These cases, although rare, have yielded important information on basic pathogenetic mechanisms in PD and suggest that failure of the UPS to degrade abnormal pro-
teins may underlie nigral degeneration and Lewy body formation that occur in PD. For example, mutations in the gene encoding parkin, which is a ubiquitin protein ligase, have been found to inactivate its ubiquitin ligating activity whereas mutations in the gene encoding UCH-L1 have been shown to lead to a decrease in ubiquitin hydrolytic activity causing a shortage of free ubiquitin resulting in a general impairment of UPS function. \(\alpha\)-synuclein has been implicated in both sporadic and familial forms of PD but whether a direct link between \(\alpha\)-synuclein and the UPS has not been firmly established, impairment of \(\alpha\)-synuclein function via mutations may dis-
turb ubiquitination of \(\alpha\)-synuclein associated proteins that may lead to a disturbance in neu-
ronal homeostasis. It has also been shown that the mutant form of \(\alpha\)-synuclein is less susceptible to degradation by the proteasome and subse-
quent aggregation may lead to secondary neu-
ronal damage by inhibiting the UPS.\textsuperscript{\alpha}

**Conclusion**

Several lines of evidence argue for a common mechanism of toxicity based on protein aggrega-
tion and the impairment of the UPS in neurode-
generation. However, it is still unclear whether protein aggregation and dysfunction of the UPS is the cause or consequence of neuronal loss in these disorders, and is still a subject of speculation and intense research interest. It may well be that pro-
teasome inhibition occurs in these diseases but the pathophysiology of the neurodegenerative process per se in each disorder is likely to be mul-
tifactorial, and not a one-hit phenomenon. Therefore a key challenge in the development of disease-modifying treatments for this group of diseases is the design of therapeutic strategies that target many different cellular pathways.